STUDIES OF FACTORS AFFECTING BIRTH WEIGHT IN PIGS.

by

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A Thesis submitted to the University of Edinburgh for the degree of Doctor of Philosophy.

ACKNOWLEDGEMENTS

I should like to express my gratitude to the Directors of the Rowett Research Institute, Sir David Cuthbertson, and after him, Dr. K. L. Blaxter, F. R. S., for permission to use the facilities of the Institute, and to the Pig Industry Development Authority, London, for supplying me with a post graduate studentship and expenses. I am very pleased to thank Dr. F. W. H. Elsley for his constant advice and encouragement during the course of my studies and Mr. I. McDonald for his help and advice with the statistical analyses of the results. My thanks are also due to Dr. A. S. Jones for the time and care he spent in reading the manuscript of the thesis, to Mr. I. Philip and his staff for their care of the animals between experiments, and to all the other members of the staff of the Institute who gave me help and advice during my studies. Finally I should like to thank my wife for her continued encouragement and also for her help in handling animals at the weekends.

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CHAPTER 1 GENERAL INTRODUCTION

The pig industry today, in common with the rest of agriculture, requires increased efficiency of production to combat rising costs and static or decreasing economic returns. There are many ways of increasing efficiency, such as improving nutrition, breeding more productive strains of pigs, providing optimal environmental conditions or reducing neonatal mortality. The studies reported in this thesis will be concerned with only one aspect of the reduction of piglet mortality, that of increasing the weight of piglets at birth.

Estimates of piglet mortality from birth to weaning vary from 13% to 25%, depending to some extent on the time of weaning. Smith (1955) estimated that losses from birth to weaning at eight weeks were in the region of 17 - 18% in Large White pigs in the Northeast of Scotland. Pomeroy (1960a) estimated losses of 26%, while figures quoted by Duncan and Lodge (1960) for British conditions vary from 18% to 25%. The losses in one herd of Large White pigs over the period May 1960 until December 1961 were estimated by Sharpe (1966) to be 24.5%. It has been estimated by the Pig Industry Develop-:ment Authority that this level of mortality represents an annual loss to the industry in the region of £12 million. The population of breeding sows in Great Britain in the period 1960-64 rose from 700,000 to 900,000 (HMSO, 1965) and if mortality were then reduced by one-:quarter this would represent an increased profit of £3 per sow per year with no increase in outlay. The situation can also be expressed by observing that 2½ million piglets die every year. Thus a reduction of the losses by one-quarter would mean that production could be maintained with 5% fewer sows (P.I.D.A., 1963).

The causes of death, both direct and indirect, in young pigs, are numerous. Sharpe (1966) has summarised the immediate causes of deaths/

deaths in the table reproduced as follows:-

Cause	% of Pigs dying
Trauma	21.2
Starvation	17.1
Runt	9.6
Septicaemia	2.7
Gastroenteritis	6.9
Cogenital Abormality	2.1
Excess pigs killed	6.8
Remainder	33.6
	100.0

In assessing the relative importance of the contributory factors, climate, age of sow, birthweight of piglets etc., Sharpe concluded that the greatest contributory factor in neonatal mortality was low birthweight, in that, while 82% of pigs weighing 400-799 g at birth died, only 7.4% of those weighing between 1500-1899 g at birth died. Similar relationships between birthweight and mortality have been reported by Jesperson and Olsen (1939), Meyer (1940), Olofsson (1950) and Lodge, McDonald and MacPherson (1961a).

Birthweight in pigs has, however, proved almost impossible to alter, in any direction. The estimates of heritability of birthweight are very low, 0-6% (Duncan and Lodge, 1960), the variation in birth-:weight being attributed almost equally to within sow and between sow variation. Neither breed, age of sow, nor litter number have a large effect on birthweight (Duncan and Lodge, 1960), while litter size has effect mainly at the two extremes, piglets in small litters tending to be heavier than in very large litters.

Unlike the sheep (Wallace, 1948) the level of nutrition during pregnancy appears to have little or no effect on the birthweight of pigs. The subject of nutrition during pregnancy was reviewed by Duncan and Lodge (1960) and from the evidence presented it can be concluded that there was at that time little evidence that birthweight could/

could be influenced by nutritional means.

Since then, there has been little further evidence indicating an effect of nutritional treatment on the birthweight of pigs. Birthweight appears unaffected by either the level of protein in the diet (Stevenson and Ellis, 1957; Boaz, 1962; Salmon-Legagneur, 1963; Clawson, Richards, Matrone and Barrick, 1963; Elsley, McDonald and MacPherson 1966; Elsley, Anderson and MacPherson, 1966) or by the quality of the protein fed (Salmon-Legagneur, 1964).

There is also little evidence of any effect of the level of energy intake affecting birthweight either when the level is constant throughout pregnancy (Self, Grummer, Hays and Spiers, 1960; Salmon-Legagneur, 1961;) or when the level is altered during pregnancy (Salmon-Legagneur, Gomez and Jaquot, 1960; Salmon-Legagneur, 1962; Elsley, Anderson and MacPherson, 1966;) However, Clawson et al (1963) and Todd (1964) considered that increased energy did increase the birthweight slightly. The interpretation of the statement that increased energy increased birthweight must, however, be made with care as in some cases the result could also be considered to mean that pathologically low levels of energy decreased birthweight. This statement is almost certainly true of the results quoted by Todd (1964). Obviously a deficiency of any nutrient if sufficiently acute will depress birthweight, but this result is of limited use if it cannot be shown that an increase of this nutrient in excess of accepted standards will increase birthweight.

Lodge (1959) reported that the level of feeding during lactation had no effect on the birthweight of the following litter and Self et al (1960) found no effect of the level of feeding during the growing period on the reproductive performance of pigs.

This brief summary of the literature has only served to reinforce the/

the view that birthweight in the pig is not readily influenced by the nutrition of the sow during pregnancy (Elsley, 1964). If then the birthweight is unaffected by nutrition or breeding, the prospects of increasing this factor seem limited.

The approach adopted in the work reported in this thesis was based on the observation by Lodge, McDonald and MacPherson (1961a) that in a group of 21 Wessex Saddleback sows, there were no significant differences in mean birthweight over the first three litters of the same sow but that there were highly significant differences ($P < 0.001$) between sows in the mean birthweight of their piglets.

Thus by comparing those animals which persistently produce litters of high average birthweight and those animals which persistently produce litters of low average birthweight, some information might be obtained on the factors affecting birthweight. In using this approach no attempt can be made to affect birthweight and furthermore it can only be used to investigate between-sow differences, leaving the problem of within-sow differences quite untouched. It is intended in this series of experiments to attempt to relate the between-sow differences in the birthweight of their offspring to some aspect of the physiology of the sow during pregnancy. The aspects which will be investigated will be those of changes in blood volume during pregnancy and the differences in glucose tolerance between sows.

CHAPTER 2. A REVIEW OF BLOOD VOLUME IN PREGNANCY AND OF THE METHODS AVAILABLE FOR ITS MEASUREMENT.

a) The volume of blood during pregnancy. Mammalian young develop in utero in order that during a very critical stage of development they should be protected from predators and from a possible hostile envir-:onment. To achieve this the parent animal must provide in the uterus an environment which fulfils the following requirements:

1. Temperature must be maintained at a favourable level.
2. Sufficient quantities of nutrients and oxygen must be available to the developing foetus.
3. Waste products of foetal metabolism must be removed.
4. The foetus must be protected from mechanical injury.

To fulfil those requirements the parent animal must make adaptations from the non-pregnant state and the efficiency with which these adaptations are made will be reflected in health and vigour of the ensuing young.

One of the major changes which occurs during pregnancy is the increase in total blood volume and the alteration in the propor-:tion of its major components, plasma and red cells.

The changes which occur in plasma volume during pregnancy are well known and all species so far examined show an increase in this parameter. The majority of studies published have concerned measurements made on human subjects.

Hytten and Leitch (1964), reviewing the subject, studied 16 papers published in the last 30 years and concluded from the evidence that, on average, human plasma volume rises to a maximum of 3900 ml 30 to 60 days before parturition and then falls to about 3600 ml during/

during the last 30 days. Using the plasma volume obtained 30 days after parturition as the non-pregnant level, they concluded that the plasma volume increase during pregnancy in humans is about 1300 ml. Hytten and Paintin (1963) examined statistically the data on 39 healthy primigravidae and determined the relationship between stage of pregnancy and plasma volume. After adjustment for height and weight the relationship was expressed by the formula

$$\text{Plasma Volume (ml)} = 2819 - 69.17W + 6.814W^2 - 0.1151W^3$$

where W = weeks of gestation between 12 and 38.

Multigravidae, it was pointed out, showed a larger increase over pregnancy than primigravidae.

Reynolds (1953) found that plasma volume in pregnant jersey cattle increased in proportion to bodyweight, and the figure for plasma volume per kg liveweight remained constant. In the sheep, Barcroft, Kennedy, and Mason (1939), using a small number of animals, found an increase of approximately 600 ml. from an initial level of 1400 ml.

The facts concerning the magnitude of the increase in the volume of red cells during pregnancy are less well established. From consideration of the published data on the changes in red cell volume during pregnancy in women, Hytten and Leitch (1964) concluded that because of differences in techniques of measuring red cell volume and differences in other variables such as treat-:ment with iron during pregnancy, no very accurate estimate of the increase in red cell volume could be made. From consideration of the twelve available papers on the subject, Hytten and Leitch, estimated that the increases in red cell volume during pregnancy over the non-pregnant values in women were in the region of 50 ml at 20 weeks, 150 ml at 30 weeks and 250 ml by parturition. In the sheep, Barcroft et al (1938) found small increases over pregnancy, while/

while in the cow, Reynolds found that red cell volume increased in proportion to bodyweight.

From these limited results it is obvious that pregnancy causes an increase in both plasma and red cell volumes, with a greater net increase in plasma volume. In humans at least it has also been shown that there is a disproportionately greater increase in plasma than red cell volumes.

These increases will provide an environment which is more conducive to rapid healthy growth of the foetus by providing:-

1. More fluid to fill the increased capacity of the vascular system provided by the gravid uterus and its contents.
2. Oxygenation of the tissues associated with pregnancy, mainly the uterus and contents and the mammary glands. Increased red cell volume will be associated with an increase in total circulating haemoglobin since, in the human at least, mean corpuscular haemoglobin concentration does not alter during pregnancy. This increased haemoglobin will provide transport for the increased oxygen requirements.

The increased oxygen required by the increasing body tissue, both maternal and foetal, could also be provided by increasing cardiac output and this does happen in the human where it increases from 4.5 litres per minute to 6 litres per minute (Hytten and Leitch, 1964); Although the mechanism controlling cardiac output, even in the normal animal, is poorly understood (Brecher and Galletti, 1963); increased cardiac output does seem to be associated with increased plasma volume, (Epstein and Ferguson, 1955; Schreiner, Freinkel, Athens and Stone, 1953). Whether the relationship is direct cause and effect is not clear, but it is possible that increased plasma volume may be associated with increased atrial filling which is in turn associated with increased cardiac output.

3./

3. Possibly increased peripheral flow to dissipate increased heat production from the growing foetuses. It has been suggested that the disproportionate increase in plasma relative to red cells in humans makes the blood less viscous, thereby reducing peripheral resistance and increasing peripheral flow (Dieckman and Wegner, 1934); Some evidence of this was reported by Bader, Bader, Rose and Braunwald (1955) who calculated that in mid-pregnancy peripheral resistance was 986 dynes/sec. cm $^{-5}$ and rose to the normal non-:pregnant value of 1250 dynes/sec. cm $^{-5}$ towards the end of pregnancy.

It is not unreasonable to suppose that increases in plasma volume, red cell volume and total circulating haemoglobin should correlate with the weight of the products of conception and possibly with the actual weight of the foetus or foetuses produced. In the pig, a polytocous animal, this should also be the case for the average birthweight of the litter provided that the numbers born are within a narrow range. In the human, Hytten and Paintin (1963) have found such a correlation between the increase in plasma volume and birthweight. They calculated that

$$\text{Birthweight (g)} = 1900 + 60I + 23S \quad (r = +0.44)$$

$$\text{where } I = \frac{\text{maximum increment}}{100}$$

$$S = \frac{\text{non-pregnant volume}}{100}$$

b) <u>The methods of measuring blood volume and the selection of a suitable method</u>. Blood is made up of two main components, red cells and plasma, and the approximate ratio of these two components in the vascular system can be obtained from the venous haematocrit. As a result blood volume can be measured either as a whole, or one component measured and the other estimated from this and the haematocrit. /

The techniques which have been used to measure the volume of blood or its components can be divided into five main categories, the first of which is now only of historical interest.

(1) Blood dilution. This method involves the measurement of the change in concentration of whole blood following the infusion of large known volumes of fluids into the vascular system. The fluids infused have included distilled water, saline and whole blood. The concentrations measured have included those of total solids, red cells and haemoglobin.

(2) Exsanguination. This method involves the bleeding out of the animal followed by the flushing out of the vascular system, usually with water. The total amount of haemoglobin recovered is determined and from this and a previously determined concen-:tration of haemoglobin in the whole blood, the total blood volume is determined.

(3) Indirect measurement of red cell volume by dilution of a known amount of red cells. A known quantity of labelled red cells are injected into the subject's blood and from the extent by which they are diluted by the subject's red cells the red cell volume is calculated.

(4) Indirect measurement of plasma volume by dilution of a known amount of test substance in the circulating plasma. This method requires the injection into the bloodstream of a known amount of test substance which mixes exclusively with the plasma but is not rapidly lost from the plasma. The extent to which a known amount of test substance is diluted gives a measure of plasma volume.

(5) Indirect measurement of total blood volume, using plasma and red cell markers simultaneously. In estimating total blood volume/

volume from plasma or red cell volume and haematocrit, a number of errors are incurred. It has been suggested by Gregersen and Rawson (1959) that the most accurate estimate of blood volume is obtained when both plasma and red cell volumes are measured simultaneously.

Present-day methods of measuring the volume of blood or its components fall into categories 3, 4, and 5, and involve the principal of dilution of a known quantity of a labelling agent which is non-toxic and should be lost very slowly from the bloodstream. Since many labelling agents are lost, either by elimination from the blood or by radio-active decay, it is often necessary to correct for losses from the blood while the label is being mixed uniformly throughout the system. Mixing is usually completed 10 minutes after injection. Provided that the label is lost from the bloodstream at a constant rate for at least 30 minutes after this, the loss during mixing can be estimated from the rate of loss during the following period.

Both red cells and plasma proteins are present outside the vascular system and some labels measure these extra-vascular components. Thus although the principle of dilution is uniform, the characteristics of each label determine the modifications to be made to the method and the interpretation of the results.

Red blood cells can be labelled with either carbon monoxide or radio-active isotopes of iron, phosphorus or chromium. Radio-:active iron, Fe 55-59, is incorporated into haemoglobin of the erythrocyte and this can be achieved only in vivo. Radio-active iron therefore must be injected into a donor and marked cells removed from the donor to be used on the test animal. This involves the cross-typing of the blood of the donor and test animals. (Hahn, Balfour, Ross, Bale and Whipple 1941 and 1942).

Radio/

Radio-active phosphorus, which also enters the erythrocyte, was used by Hahn and Hevesey (1940), who specified the use of a donor animal. Anderson (1942) described a method for the in vitro labelling of red cells with phosphorus. Radio-active phosphorus is lost from erythrocytes at approximately 6% per hour in man, dog and rat (Gregersen and Rawson 1959). A correction for loss during the mixing period must be made and this requires serial blood sampling after the injection of this marker.

Sterling and Gray (1950) described a method for the estimation of red blood cell volume using erythrocytes marked with radio-active chromium (Cr^{51}). This marker, in the form of $Na_2 Cr^{51}O_4$ is taken up readily by erythrocytes in vitro and shows no significant loss to the plasma in 24 hours. One sample of blood taken after mixing is therefore sufficient for accurate measurements of red cell volume.

The carbon monoxide method of measuring red blood cell volumes requires either the inhalation of a known quantity of CO or the injection of previously saturated donor red cells (Root, Roughton and Gregerson, 1946). (Nomof, Hopper, Brown, Scott, and Wennesland, 1954). concluded that: "This method is now thought to over-estimate red cell volume by about 12-14%, the CO being absorbed by non-circulating substances having an affinity to CO".

The labels used to measure plasma volume can be divided into three categories - 1) radio-active isotopes, 2) dyes, and 3) substances of very high molecular weight.

The principal requirement of a label used for plasma volume determination is that it should have either a very high molecular weight or be attached to a molecule of very high molecular weight so/

so that it will not be lost through the capillary walls to the extra-:cellular fluid. The labels in groups 1 and 2 are therefore invariably attached to protein molecules such as albumin or fibrinogen which reduces the loss to the extra-cellular fluid but does not eliminate it. Attachment of the label to the protein may be done before injection of the label into the bloodstream or may take place immediately on injection.

1) The most commonly used radio-active isotope in plasma volume determinations is iodine I^{131} (Gibson, Seligman, Peacock, Aub, Fine and Evans, 1946) and is injected in the form of radio-:iodinated serum albumin, (R.I.S.A.) which can be prepared, but is available commercially. Commercial preparations of R.I.S.A., contain 2-5% of free iodine I^{131} (Crane and Adams 1954) and a foreign protein. Dilute solutions of R.I.S.A., are progressively adsorbed onto glassware (Gregersen and Rawson 1959). Radio-:active chromium (Cr^{51}) in the form of $Cr^{51}Cl_3$ when injected in saline solution becomes 98% bound to the plasma proteins (Gray and Sterling 1950; Gray and Frank 1953). The method, however, has been little used and requires further investigation.

2) Dyes which are used for plasma volume determinations are all injected in water or saline solution and rapidly become bound firmly to the plasma proteins. The dyes can be divided into two classes, the blue and the red dyes, but the red dyes are rarely used now since their region of peak optical density corresponds with that of haemoglobin, a common contaminant of plasma samples. Since dyes are attached to the plasma albumin which is lost through the capillary walls to the extra-cellular fluid at an appreciable rate, correction must be made for loss of dye during mixing.

The concentration of dyes in plasma is always measured optically either in the plasma or after extraction. Plasma itself has/

has an inherent colour and this affects the wavelength of peak optical density of the dye in plasma, both between species (Allen, Ochoa, Ross and Gregersen, 1953) and within species (Talbot and Swensen, 1963). The extraction of dye from the plasma would overcome this difficulty but methods of extraction so far evolved (Campbell, Frohman and Reeve, 1958) do not recover a constant proportion of the dye from the plasma.

3) Materials which are not normally present in the blood and which have a high molecular weight are used as tracers. These molecules are of such high molecular weight that they are not lost through the capillary walls and therefore measure true plasma volume. The materials used are purified high molecular weight dextrans (Craig and Waterhouse, 1957; Verel, 1958); or iron-:dextrans (McKenzie and Tindle, 1959; Butler and Watkins, 1966).

In selecting a suitable method of measuring the volume of blood or one of its components, two decisions had to be made; whether to measure plasma or red cell volume and whether to use radio-active or non radio-active labels.

Since a relationship had been reported between the increase in plasma volume during pregnancy and the birthweight of the child in humans (Hytten and Paintin, 1963) it was considered desirable to measure plasma volume. The choice between the use of a radio-:active label or a non-radio-active label was made largely on the basis of cost. In the studies envisaged it was hoped to use a large number of animals, and since radio-active labels are expensive both in terms of cost of materials and the total loss of contaminated carcasses, the expense was deemed prohibitive, at least in the initial stages. Of the non-radio-active labels used in plasma volume determinations the blue dye Evans blue (T. 1824) was the best documented and it was decided to use this label.

c)/

c) The estimation of plasma volume using Evans blue. Evans blue was introduced as a label for plasma volume determinations by Dawson, Evans and Whipple in 1920. It is an azo-dye, and its peak optical density occurs at 620 mμ. At this wavelength errors due to the presence of haemoglobin in the plasma are considerably reduced. On injection into the bloodstream the dye is firmly and selectively bound to the plasma albumin (Rawson 1943). The dye is non-toxic (Gregersen and Rawson, 1959) and it does not cross the placenta of the cow (Reynolds 1953).

The dye is lost from the bloodstream at an appreciable rate in all species so far tested (Gregersen and Rawson 1959) while the rate of loss was reported by Talbot and Swensen (1963) to be 20% per hour in the pig. The rate of loss of Evans blue from the bloodstream was considered by Gregersen and Rawson (1959) to be constant for a period of one hour in the human and the dog, and to conform approximately to a first order rate reaction. This was also considered to be the case in the pig by Talbot and Swensen (1963). Thus by calculating the regression of the logarithm of Evans blue concentration on time during the period 10-60 minutes after injection of the dye and extrapolating back to zero time (the time of injection of dye into the animal) the loss of dye from the circulation during the mixing phase (0-10 minutes) can be accounted for. This procedure depends upon the assumption that the rate of loss of dye from the blood in the mixing period is identical with the rate occurr-:ing during the following 50 minutes. Allen and Gregersen (1953) showed that the volume distribution of Evans blue was consistent in the dog over a range of dye concentrations of 8-1260 μg / ml plasma. This situation is unlikely to occur if there is a more rapid loss of dye during the mixing period. In species where the rate of loss of Evans blue from the plasma is low, the losses during mixing are slight and the/

the concentration of dye in plasma 10 minutes after injection will give an acceptable estimate of plasma volume (Noble and Gregersen 1946).

The concentration of Evans blue in a fluid is determined photometrically. Therefore, any substance present in the fluid which absorbs light at 620 m μ will cause errors in the estimation of the concentration of the dye. Variations in inherent plasma colour appears to be especially marked in pigs, and Talbot and Swensen (1963) recommended that the Evans blue should be extracted from the plasma before being measured. However, the method of extracting Evans blue from the plasma (Campbell et al, 1958) recovers a variable quantity of the dye present in the plasma (94 - 98%), and extraction methods were found unsuitable by Hytten (personal communication). To overcome this difficulty standards of Evans blue in plasma should be made up for each animal from a plasma sample collected immediately before the injection of the Evans blue. To reduce errors caused by the individual spectral characteristics of plasma samples still further, it is advisable to make up the plasma standard to a concentration of dye in plasma similar to that which is expected in the animals plasma after injection of the dye.

Alimentary lipaemia also causes interference in the estimation of Evans blue in plasma. This can be overcome by withholding food from the subject for 12 hours previous to the determination, and also by determining the concentration of Evans blue in serum rather than plasma.

d) The estimation of total blood volume from plasma volume and haematocrit. The true total blood volume can be considered as the sum of the measured plasma volume (PV) and the measured red cell volume (CV) both having been determined simultaneously. An

estimate/

estimate of total blood volume can be derived from the measured plasma or red cell volume and haematocrit, using the equation

$$\text{Total blood volume} = \frac{PV}{(100-H)} \times \frac{100}{1}$$ where H = venous haematocrit.

This equation, however, only gives an approximation of true total blood volume since the value of H obtained using normal clinical procedures requires considerable modification before it gives a true picture of the ratio of red cells to plasma throughout the vascular system.

In the first instance the haematocrit as determined by centrifugation at moderate 'g' (3000-4000) is not identical with the true red cell percentage in the sample. Some plasma is always left within the column of red cells and is generally referred to as the "trapped plasma". This is expressed as the percentage of the length of the red cell column which is actually plasma.

The magnitude of the error is dependent on the force which is applied to the sample, expressed as the relative centrifugal force (RCF), the time during which this force is applied (T), and also upon the haematocrit of the sample. It was suggested by Dole and Cotzias (1951) that a multiple of RCF and time would be a useful concept in resolving differences of both RCF and time of centrifugation reported by different authors. However, both Hlad and Holmes (1953) and Hodgetts (1959) have reported that the use of a measure including RCF and time, impulse, does not completely describe the relationship between time RCF and the trapped plasma in blood samples of the same haematocrit.

It has been shown (Owen and Powers, 1953; Hlad and Holmes, 1953) that the distribution of trapped plasma within the red cell column is not uniform, the amount of trapped plasma per unit volume of red cells being greater towards the "buffy coat". Thus differing haematocrits will contain differing percentages of trapped plasma. /

plasma. Hodgetts (1959) suggested that since the RCF applied to a haematocrit tube was conventionally measured at the end of the tube farthest from the axis around which it was revolving, then the mean RCF over columns of red cells of differing lengths would become less as the column became longer and the "buffy coat" was closer to the axis. In confirmation of this she showed that the amount of trapped plasma increased linearly with increasing haematocrit.

These various factors controlling trapped plasma have made it impossible to formulate a standard correction factor. The estimates made of trapped plasma vary considerably depending upon the method used to determine the plasma in the red cell column and the conditions under which the centrifugal haematocrit was measured. In some instances authors have described the methods by which the centrifugal haematocrit was obtained in terms of revolutions per minute of the centrifuge head, without giving the radius of the head. However, the average red cell column is thought to contain about 3 - 5% of plasma although estimates vary from 12% after an RCF of 2400 'g' for 30 minutes in the sheep (Duthie 1961) to less than 2% after 5600 'g' for 30 minutes in the human (Jackson and Nutt 1951). These figures, however, are only average figures and Owen and Powers (1953) reported that in a series of samples from 39 subjects the mean trapped plasma after an RCF of 2200 'g' for 60 minutes was 3.79% $\pm$ S. E. 0.70%. The individual estimates ranged from - 2.89% to 7.9%.

No estimate of the trapped plasma has been made upon pigs' blood and such are the difficulties of extrapolating an estimate of trapped plasma from one set of experimental conditions to another, coupled with between-species differences, that any real advantage in adopting such an estimate appeared doubtful. The only useful

estimate/

estimate of trapped plasma for use in the present series of experiments would be one derived for each individual determination of blood volume, especially since there have been no investigations on the effect of advancing pregnancy on the trapped plasma fraction.

The use of the venous haematocrit to estimate toal blood volume from plasma or red cell volume depends on the assumption that the haematocrit is uniform throughout the body. In the past, when simultaneous estimates of red cell and plasma volume were made on the same animal, it was generally found that the sum of the measured red cell and plasma volumes was less than that of total blood volume obtained from plasma volume and haematocrit (Gregersen and Rawson, 1959). It is now considered that this discrepancy is due to the lack of uniformity of the haematocrit throughout the body. Allen and Reeve (1953) have shown that in the splenectomised dog, hepatic and renal bloods contain 31% and 49% less cells than the jugular blood and that this accounts for 60% of the difference between the estimated and measured total blood volumes in splenectomised animals, and probably in species such as the human where the spleen does not function as a store for red blood cells.

However, the belief that the haematocrit is not uniform throughout the body because estimates of blood volume based on plasma volume and haematocrit do not tally with measured blood volume depends upon the assumption that the method used to measure plasma volume does not over-estimate it. Andersen (1962) found that measurements of plasma volume using I^{131} labelled gamma globulins were, in humans, on average 2.5% less than those made using albumin labelled with I^{131} or Evans blue. He also pointed out that estimates of plasma volume using high molecular weight dextrans were also lower/

lower than estimates using labelled albumins and suggested that labelled albumin was not lost from the circulation at a uniform rate, and therefore plasma volume was over-estimated. It was not, however, claimed that the suggested over-estimate of plasma volume by labelled albumins accounted for the whole of the discrepancy between estimated and measured total blood volume.

The difference between the venous haematocrit and the whole body haematocrit is generally referred to as the 'F' cells correction which is expressed as the ratio of the whole-body haematocrit to the venous haematocrit corrected for trapped plasma. In those species where the spleen is not known to act as a reservoir for red blood cells, the human, rabbit and cow, the 'F' cells correction is usually of the order of 0.9. (Table 1).

Those species where the spleen is known to act as a reservoir for red blood cells which can contract or dilate with the animal's emotional state, present a very difficult problem in obtaining a useful 'F' cells correction. The species where the spleen is known to have this ability are the dog (Barcroft and Stevens, 1927; and Reeve, Gregersen, Allen and Sear, 1953;) and the sheep (Turner and Hodgetts, 1959). In these species when the animal is excited or given an injection of adrenalin the spleen contracts, ejecting into the circulation blood which contains a very high proportion of red blood cells. Conversely, when the animal is relaxed or under nembutal anaesthesia, the spleen dilates and fills with blood which contains a higher percentage of red blood cells than the venous blood. This contraction or dilation of the spleen will affect the venous haematocrit since red cells are being withdrawn from or injected into the circulation, while the volume of circulating plasma remains unaltered. The total volume of red cells in the circulatory system, however, does not alter and therefore, the relationship between/

TABLE 1. The effect of the spleen on the 'F' cells correction in several species.

Author	Animal	'F' cells mean ± S. D.	Spleen
Chaplin et al (1953)	Human	0.91 ±0.026	present
Armin et al (1952)	Rabbit	0.85 ±0.034	present
Marcilese et al (1966)	Cow	0.94 ±0.027	present
Menzies et al (1965)	Sheep ψ	1.01 ±0.060	present
Hodgetts (1961)	Sheep ψ	1.22 ±0.092	present
Hodgetts (1961)	Sheep ψ	0.81 ±0.023	present + adrenalin
Hodgetts (1961)	Sheep ψ	0.83 ±0.023	absent
Reeve et al	Dog ψ	0.98 ±0.058	present
Reeve et al	Dog ψ	1.10 ±0.051	present + nembutal
Reeve et al	Dog ψ	0.90 ±0.008	absent

ψ Species in which the spleen acts as a red cell store.

between venous haematocrit and whole body haematocrit, the 'F' cells correction, must alter with changes in the state of the spleen.

The data set out on Table 1 indicates the magnitude of the 'F' cells correction in a number of species and the magnitude of the variations which can occur in the correction of those species, where the spleen is known to act as a red cell store.

An estimate of the 'F' cells correction has been made in the pig, by Talbot and Swensen (1963). These authors used baby pigs, either untreated or treated with iron dextran while plasma volume was measured using Evans blue and red cell volume using Cr^{51}. They found that the 'F' cells correction in the treated group was 0.74 ± 0.07 and for the untreated group was 0.71 ± 0.08. This correction is very much greater than any reported in other species and since only sparse details of the methods used and the treatment of pigs during actual experiments were given, these results must be treated with some reserve.

The use of an 'F' cell correction in the present series of experiments may be further complicated in that pregnant animals are being used. In pregnancy both the uterus and the mammary glands increase greatly in size and hence require a very much greater blood supply. This could well upset the balance between the venous haematocrit and the whole body haematocrit thereby altering the 'F' cells correction. Little work on this subject has been published, but Pritchard and Rowlands (1964) reported that the 'F' cells in non-pregnant women were identical with that in pregnant women at full term (0.89). The variability in the pregnant women was, however, almost 50% greater than in the non-pregnant controls.

Since/

Since the constancy of the 'F' cells correction in a species appears to depend on whether the spleen acts as a red cell store, and no work on this subject has been reported for the pig it would be unwise to adopt any of the reported 'F' cells corrections. Any estimate of total blood volume from plasma volume in the pig can only be made after a study of the constancy of the venous haematocrit under the varying conditions of emotional stress in the experimental animal. It must also be pointed out that the use of an 'F' cells correction, however carefully derived, will only reduce the magnitude of the error in estimating blood volume from plasma volume and haematocrit, and will not reduce the variability of that error.

CHAPTER 3. STUDIES ON THE MEASUREMENT OF PLASMA AND BLOOD VOLUME IN SOWS.

Section 1 An indwelling catheter for use in pigs

a) The need for a catheter and the selection of a suitable site.

Blood samples are not readily obtained from the pig. The animal is intractible, difficult to restrain, and most of the blood vessels are covered by a thick layer of fat. The most common sites for obtaining blood samples are:-

(a) from the ear veins.

(b) from the coxygeal artery and vein after the removal of a portion of the tail.

(c) from the anterior vena cava (Carle and Dewhirst 1942).

These methods have limited application. They all cause a certain amount of discomfort to the pig both by the puncture of skin and the restraint necessary. It is not always possible to withdraw large quantities of blood rapidly from the ear and tail, while the withdrawal of samples from the vena cava in the conscious pig requires consid-:erable skill. Moreover the samples from the tail are not uniformly either arterial or venous while haemolysis occurs readily in samples from both tail and ear. In addition, none of the sites mentioned are very suitable for the infusion of substances intravenously. On the basis of these considerations it was decided to attempt to establish polyethylene catheters in the pigs.

Very little work on the establishment of indwelling catheters in pigs has been reported. Folley and Knaggs (1966) described a method of establishing a catheter in the external jugular vein of the sow. This involved the blunt dissection of the vein and the insertion of a poly-:ethylene catheter directly into the vein. The catheter was inserted in the direction of the heart. While not in use the lumen of the catheter was filled by a nylon stylette. These catheters remained

patent for periods of up to five days. A catheter can also be established in the femoral or saphenous vein of the pig, and using silicone rubber tubing these catheters may remain patent for four to five weeks (Care, Personal Communication, 1966). Both of these methods require a considerable amount of surgery which is both difficult and time-consuming. Moreover under the conditions in which the pigs used in the present studies were kept, it would have been difficult to prevent infection of the wound. After consideration of these factors, it was decided to attempt to establish catheters in the ear veins of the experimental animals, since these veins were accessible and less likely to become infected through contact with faeces.

b) The administration of the anaesthetic. The ear veins of sows are relatively small vessels and the insertion of a catheter into a vein is a delicate procedure. To ensure that the sow did not move at a critical stage of the operation it was considered necessary to use general anaesthesia during the operation. It was further decided to use gaseous anaesthesia as otherwise an ear vein would have had to be used to give an intravenous injection. The gas selected for use was Trichloro-ethylene ψ.

Initially the gas was administered from a container with holes punched in the bottom. A pad of cotton wool soaked in 'Trilene' was placed in the bottom of the container. Adhesive bandage was wound around the top of the container so that when it was placed over the animal's snout the bandages formed a gas seal between the container and the snout. Thus the animal was forced to breathe through the holes in the bottom of the container and the 'Trilene'-soaked pad.

While anaesthesia was being induced the animal was restrained in a crate, and further restrained using a 'twitch' (Miller and Robertson, 1947). The use of a twitch was discontinued since it prevented/

ψ 'Trilene' I.C.I. Ltd.

FIGURE 1. The restraining yoke.

prevented a proper gas seal. A large metal yoke was then designed and this proved very satisfactory (figure 1)

In the early stages of the study a number of animals stopped breathing and had to be resuscitated by closing the mouth and one nostril and blowing through the other nostril to expand the lungs. This process of artificial respiration was continued until the animal revived. It was found, however, that if the animal was laid flat on its side with its legs outstretched no troubles of this nature arose.

To obtain a more rapid induction of anaesthesia, and better control thereafter, a mask was designed using a longer container. Air was drawn into the mask through an inward facing rubber spear valve in the base. The air then passed through a pad of cotton wool soaked with 'Trilene', supported on a metal grid, immediately above the spear valve. The air-'Trilene' mixture was then inhaled by the animal. Exhaled air escaped from the mask through an outward facing rubber spear valve between the lip of the can and the cotton wool soaked in 'Trilene'. In this way the bulk of the exhaled air was prevented from passing throught the 'Trilene'. The gas seal between the surface of the animal's snout and the lip of the can was made of foam and rubber, which was more satisfactory than adhesive bandage.

c) <u>The insertion of the catheter</u>. After the animal had been fully anaesthetised, the ear was swabbed with decolourised iodine solution and a suitable vein was chosen. The choice of vein was found to be very important since it was not possible to thread catheters sufficiently far down some veins. The ideal vein ran out of the centre of the base of the ear. Those veins which ran around the periphery of the ear, and turned sharply, were rarely suitable, regardless of size, as only in about 30% of cases could the catheter be threaded farther than about 4" into the vein. Catheters in this position rarely remained patent

for/

FIGURE 2. Showing a sow's ear with a venous catheter secured in place by two Michel's clips with, between them, adhesive plaster rolled around the catheter.

for more than a few hours. The selected vein was then partially excised using a fine scalpel and then stasis was applied by placing a rubber band around the base of the ear. At this stage a hypodermic needle was inserted into the vein and the catheter threaded through this. A 15 gauge needle was found to be the most suitable as this was the largest size which could be used without damaging the vein. The needle was then withdrawn over the free end of the catheter which was then secured with Michel's clips and adhesive bandages (figure 2).

The catheter itself consisted of 24" of polyethylene tubing whose outside diameter was approximately 0.05" ¥ During the insertion of the catheter, and at all times thereafter when the catheter was not in use, the lumen of the catheter was occupied by a plastic stylette. This protruded beyond both ends of the catheter and had a knot tied in it at the outside end.

It was found by experience that catheters were more successful if they were inserted with a length at least 16" - 18" within the venous system. In this position the tip of the catheter lay in the jugular vein. This was confirmed by X-ray photography.

The free end of the catheter usually reached to a point 1" - 2" beyond the tip of the animal's ear, leaving approximately 5" - 6" of catheter free. This was found to be the most suitable position as while a blood sample was removed using a syringe in the right hand, the ear could be held between 2nd and 3rd fingers and the tip of the catheter between the thumb and 1st finger of the left hand. The syringe was connected to the catheter using a 19 gauge hypodermic needle which fitted snugly into the internal diameter of the catheter.

d) Procedures used to maintain the catheters patent. Once the catheter/

¥ Portex Plastics Ltd (pp. 90)

catheter was in position it was maintained patent by removing the stylette every two days and flushing out the catheter with heparinised saline (100 i.u. / ml.) and then inserting a fresh sterile stylette. Catheters treated in this way remained patent for 3 to 4 weeks but this length of time proved to be inadequate.

It was found by experience that usually only one vein in each ear was suitable for cannulation, and each vein could only have a catheter inserted on one occasion. Thus if it was wished to maintain a patent catheter in an animal throughout pregnancy (16 weeks) each catheter had to remain patent for at least 8 weeks.

Provided the adhesive bandages were checked daily, no difficulty was found in keeping the catheters in position for long periods but it was found very difficult to keep the catheters patent for long periods. Over a period of time the following method evolved. If blood samples could not be obtained from the catheter, the animal was persuaded to move its head about while suction was applied to the catheter and this frequently freed the obstruction. If this treatment was unsuccessful the catheter was left and the same procedure repeated on the following day. On several occasions the catheters were found to be patent on the second day without further treatment. If, however, this treatment was unsuccessful the stylette was returned, the catheter withdrawn slightly, and then pushed back to its original position or beyond. The stylette was then removed and suction applied.

If this procedure failed the animal was anaesthetised and the catheter checked to see whether the obstruction in the catheter had been freed in the process. If the catheter was still blocked, all the bandages were removed and a blunt needle with the wall ground down as thin as possible, to prevent damage to the site of entry, was slipped over the catheter into the vein. The blocked catheter was

then withdrawn and a fresh catheter, which had been well lubricated with liquid paraffin, was inserted into the vein.

On first attempts at replacing the catheter, about 70% were reinserted without difficulty and ran successfully provided the tip lay some 1" or 2" nearer the heart. In the remaining 30% resistance to the returning catheter was encountered but with the rigidity imparted by the stylette a twisting motion could be employed and in about half of these cases the obstruction could be passed. If however it proved difficult to withdraw the stylette afterwards it was essential to use the catheter within a few hours as such catheters were unlikely to run for longer than one day.

Using these procedures it was found possible to maintain the catheters patent for as long as 22 weeks and it might well be possible to extend this period if more time were spent on the catheters, for example by flushing out twice daily. Some workers have reported that they have maintained portal vein catheters patent in ruminants for periods up to 73 days (Connor and Fries, 1960) using large quantities of heparin intravenously, or for 160 days (Moodie, Walker and Hutton, 1963) when the catheter was flushed at least twice per day throughout. The success of the catheters reported here is due not only to the care and attention given to them but also to the discovery that a blocked catheter can be replaced readily in the same vein.

The work described on the preceding pages has shown that catheters can be inserted readily into pigs without major surgery or post-operative infection, and that they can be maintained patent for long periods with moderate care and attention. This technique should enable studies involving sampling of blood or infusion of substances into the pig's vascular system to be carried out quite simply and easily.

Section II/

Section II The measurement of plasma volume in pigs using Evans blue.

a) The method used. The method adopted to measure plasma volume using Evans blue was basically that outlined by Gregersen and Rawson (1959). Powdered Evans blue[ψ] was made up in a 1.5% (w/v) solution with distilled water. This was known as the injection solution. Every two weeks 1 ml of the injection solution was made up to 50 ml with distilled water to make the 'standard solution'. The standard solution was used on the day of the determination to make up the plasma standard. This was a known concentration of Evans blue in a sample of plasma obtained from the pig under experiment on the morning of the experiment before the Evans blue was injected.

All determinations of plasma volume were made at 9.30 a.m., on animals which had not been fed since the previous afternoon. The sampling of blood and the injection of Evans blue were carried out in a metabolism crate in which the animal had spent the previous night.

A disposable plastic syringe was weighed empty and then re-:weighed containing a quantity of 1.5% Evans blue in it.

The bandages securing the end of the catheter were removed and the stylette withdrawn. The catheter was then flushed out with heparinised saline (0.9% NaCl and 100 i.u. heparin / ml). Before any blood sample for analysis was taken, a small quantity of blood (1 - 2 ml) was withdrawn from the catheter and discarded to remove all the saline solution from the catheter. All blood samples for analysis were collected into fresh disposable syringes.

A 20 - ml basal sample was removed from the animal and allowed to run into a heparinised, universal sample bottle. The bottle was immediately rotated gently to ensure the mixing of the heparin through the blood.

The/

ψ B.D.H. Ltd.

The entire contents of the syringe which contained the Evans blue were then injected into the animal. To ensure complete emptying, the syringe was flushed out four times with the pig's own blood, followed by 5 ml. of heparinised saline.

A stop watch was started as injection began, and 10 - ml. samples of blood were removed at 10, 20, 30, 40, 50 and 60 minutes after injection. After each sample the catheter was flushed out with 2 ml. heparinised saline.

A small quantity of blood was removed from the basal, 30 and 60 - minute blood samples for haematocrit and haemoglobin determin-:ations, and the remainder placed in a centrifuge and subjected to a force of 3000 'g' for 30 minutes, after which the plasma was separated from the red cells.

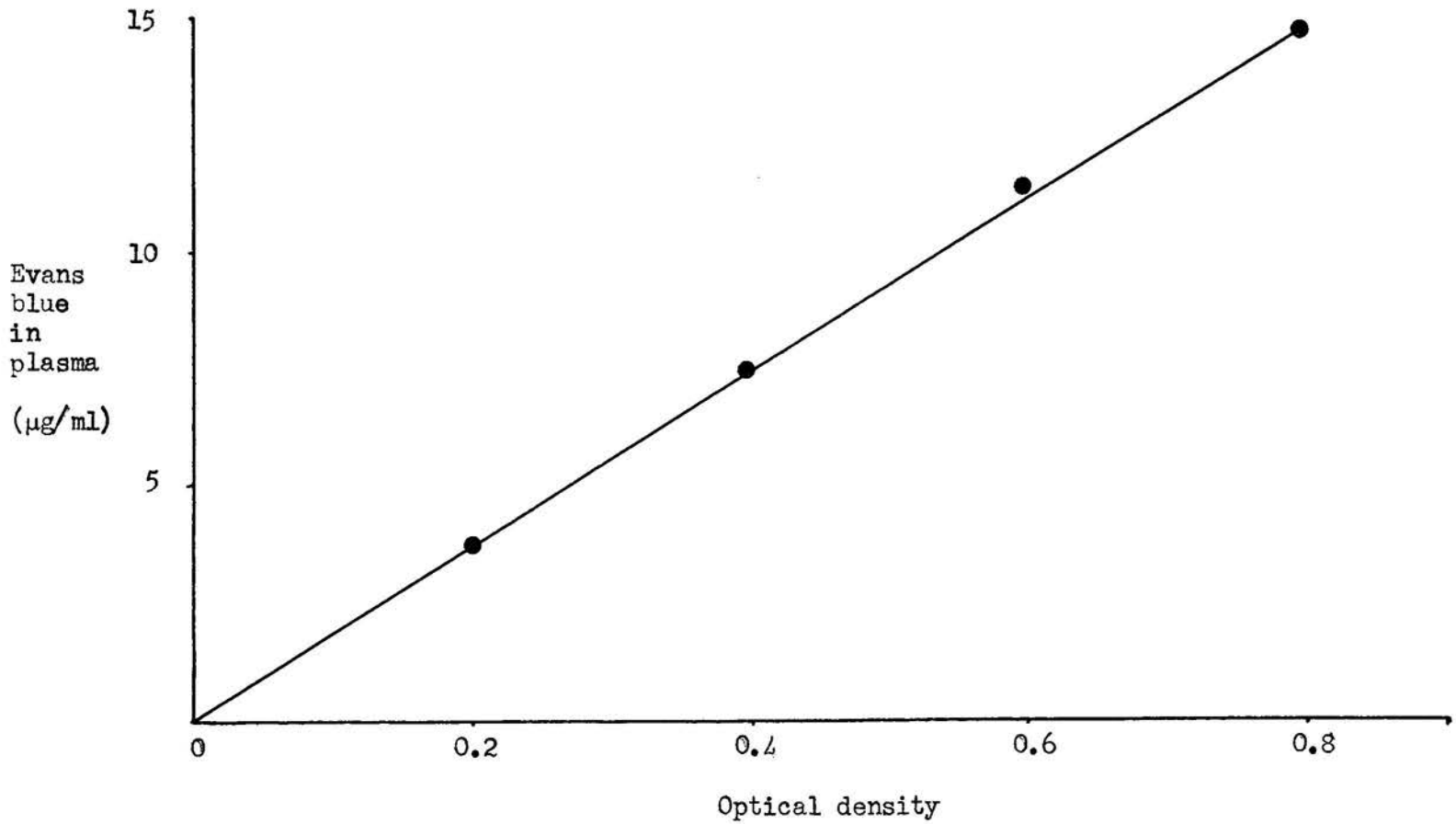
The plasma from the basal sample was divided into two portions; 5 ml. of plasma to provide a standard and the remainder to provide a blank. The standard was prepared by adding 0.06 ml. of 0.030% Evans blue solution to 5 ml. plasma. The concentration of Evans blue in the standard was then 0.00357 mg / ml.

The concentration of dye in the plasma of the samples taken after Evans blue injection was found by comparing the optical density of these samples with that of the standard.

The optical density of the plasma + dye solutions was measured at 620 mμ in 1 cm matched glass cells using a spectrophotometer (Unicam S.P. 600) which was set to read optical density 0 with the plasma blank in place.

The calculation of the concentration of Evans blue in plasma was based on the assumption that solutions of Evans blue in plasma obey Beer - Lambert's Law. To verify this, solutions of Evans blue in plasma were made up to cover a range of concentrations from 0.0036 to 0.01 mg Evans blue per ml plasma and the optical density of the solutions/

FIGURE 3. **The relationship between the concentration of Evans blue in plasma and the optical density of the plasma.**


15
10
5
Evans blue in plasma (µg/ml)
0
0.2
0.4
0.6
0.8
Optical density

solutions measured. The optical density at 620 mμ of plasma + Evans blue was found to be directly related to the concentration of Evans blue present in the plasma (figure 3).

The amount of Evans blue injected into the animal was originally designed to make the optical density of the plasma-dye solutions within the range 0.2 - 0.7 which is the region of maximum sensitivity of spectrophotometers (Varley 1963).

The dose required, 0.3 mg Evans blue /kg bodyweight, was however found to cause slight shock in the animals and was reduced to 0.15 mg/kg bodyweight. Using this quantity of dye, no further signs of shock were noticed, but an optical density of 0.15 - 0.2 in the plasma samples (using 1 cm cells) had to be accepted. The concentration of the dye in the plasma standard was also lowered to give an optical density in this range.

Since it was vital that a known quantity of dye should be injected into the animal, the greatest care was taken to see that no dye was lost from the injection system. When the syringe containing the weighed quantity of Evans blue was attached to the catheter, blood was drawn into the syringe. This immediately showed up any leaks in the system which could then be corrected. The mixing of blood with the Evans blue solution also prevented adhesion of the dye to the walls of the catheter, presumably because the dye adhered preferentially to the plasma albumin fraction.

The concentration of the dye in the plasma at zero time was estimated using the method of back-extrapolation of the rate of loss of dye from the plasma during the period 10 - 60 minutes after injection. The regression of the logarithm of the concentration of dye in the plasma on time was calculated using the method of least squares.

b) <u>The repeatability of the method under different circumstances.</u> In order to determine the degree of repeatability of the method as described, /

described, a number of determinations of plasma volume were carried out on successive days (Table 2). This demonstrated that the method was repeatable, the second determination being 100.78% of the first, with a standard deviation of $\pm$ 2.56%.

Since the concentration of Evans blue in plasma achieved in vivo between animals was not constant it was considered necessary to test the repeatability of the method when the dose was altered.

A catheter was established in one animal and measurements of plasma volume were made over a period of eleven days using different quantitites of injected dye. To prevent any build-up of dye in the plasma when so many determinations were to be made on the same animal, the interval between determinations was increased to four days after the second determination.

The results of this experiment, which are shown in Table 3 indicated that the estimate of plasma volume is not markedly affected by the dose, at least within the range of doses tested in this experiment. The estimated plasma volume did alter, but these alterations were independent of dose. The small alterations may be explained by one or both of the following facts:-

1) The animal was confined to a metabolism crate for the entire period of the test; Taylor, Erickson, Henschel and Keys (1945) observed that in humans plasma volume decreased by 15% after bed rest of 3 weeks.

2) A total of 500 ml. whole blood were removed from this animal between day one and day ten of the experiment, and this is equiva-:lent to approximately 300-350 ml plasma.

The method was also tested to ascertain whether the injection of dye and the removal of post-injection blood samples through the same catheter introduced any error into the determination. For instance it was feared that a very small quantity of dye might be left adhering to the catheter during injection, and that this dye might contaminate some

of/

TABLE 2. The repeatability of estimates of plasma volume in the pig using Evans blue

Test	Interval between determn. (days)	Determination 1	Determination 2	Difference (ml)	Difference (%)
1	1	8493	8178	-315	-3.70
2	1	6876	6977	+101	+1.40
3	2	5597	5717	+120	+2.20
4	2	5264	5409	+145	+2.70
5	1	6473	6426	- 47	-0.72
6	2	5909	6075	+116	+2.81

TABLE 3. The effect of dose of Evans blue on the estimate of plasma volume.

Day	Dose (mg)	Conc. in plasma (ug/ml)	Estimated plasma volume (ml)	Error 1 (ml)	Error 2 (ml)	Error 1 (%)	Error 2 (%)
1	43.336	4.93	8863	-	-	-	-
3	21.861	2.52	8693	170	170	1.90	1.90
7	21.497	2.55	8430	263	433	3.00	5.10
11	55.820	6.72	8307	123	556	1.40	6.30

Error 1 = error between consecutive estimates.

Error 2 = error between estimation and estimation on day 1.

of the post-injection blood samples.

One animal was prepared with a catheter inserted in a vein in both ears. The catheters were of different internal lengths (14" and 18") to ensure completely different sampling sites. Basal blood samples were removed from both catheters before Evans blue was injected through the longer catheter. As it proved impossible to remove samples from both catheters simultaneously, the longer catheter was used at approximately 10, 20, 30, 40, 50, and 60 minutes post-injection and the shorter catheter at 6.5, 16, 25, 35, 45 and 55 minutes post-injection. Standards and blanks were made up from each basal sample and used to determine concentration of dye in the appropriate series of samples. There was no difference in the estimated plasma volume between catheters (Table 4).

The effect of 'Trilene' anaesthesia on the repeatability of the method was also investiaged since the possibility of measuring the plasma volume under anaesthesia in intractable animals was consid-:ered. In anaesthetised animals, the loss of Evans blue from the plasma was not found to be a first order rate reaction, the concen-:tration of dye in plasma fluctuating widely and apparently randomly. No estimates of plasma volume were made while the animal was anaesthetised and only one animal had to be discarded because of intractability.

c) <u>The pattern of the rate of loss of Evans blue from the plasma in the pig and its effect on the estimate of plasma volume</u>. The validity of the estimate of the concentration of Evans blue in plasma at zero time, depends upon the assumption that the rate of loss of dye from plasma is uniform throughout the mixing period and for a sufficient time thereafter to estimate the loss of dye from the plasma during mixing. Talbot and Swensen (1963) claimed that the rate of loss of Evans blue from the plasma was, in the young pig, a first order rate reaction/

TABLE 4. Plasma volume estimated from samples taken from different sites on the animal.

	Long (injection) catheter	short catheter
Plasma volume (ml)	8863	8836
Haematocrit (%)	31.0	31.0
Error (ml)		-27

reaction, and that the method of back-extrapolation was valid.

The data from the first 21 estimates of plasma volume were examined to determine whether the rate of loss of Evans blue from the plasma was a first order reaction in the adult pig. A visual examination of the plots of the logarithm of the concentration of Evans blue in plasma with time indicated a curvilinear relationship. This was tested statistically by considering the standard period of 10 - 60 minutes after injection as three periods, 10 - 30 minutes, 20 - 30 minutes and 40 - 60 minutes. These periods were chosen because they were indicative of the changes in slope of the plot.

The 21 determinations were divided into two groups; determin-:ations 1 - 14 and determinations 15 - 21. During the period when determinations 1 - 14 were performed, methods of handling animals and blood samples were being developed, but determinations 15 - 21 were performed when methods had been, to a large extent, worked out. Statistical analysis of the mean slopes of the logarithm of concentration with time indicated no significant difference between the two groups and subsequent analysis was carried out on the basis of all 21 determinations together and on the last 7 determinations alone. The last 7 determinations were analysed separately because, although there was no significant difference between groups, random errors due to excitement of the animals and mishandling of blood samples would be reduced. The results which are shown in Table 5 indicate that the slope of the regression of the logarithm of concentration varies with time and that the slope in any one period is significantly different from the slope in the other two periods. It can also be shown that the slope becomes progressively less steep with time, the slopes in periods 10 - 30, 20 - 30 and 40 - 60 being 270×10^{-5}, 200×10^{-5} and 70×10^{-5} respectively.

These determinations were all made when the dose of Evans blue injected/

TABLE 5. The loss of Evans blue from plasma in the pig.

Comparison of slopes	Significance of difference on	
	all 21 determinations	determinations 15-21
10 - 30 mins. v. 20 - 30 mins.	$P < 0.001$	$P < 0.01$
20 - 30 mins. v. 40 - 60 mins.	$P < 0.05$	$P < 0.01$
10 - 30 mins. v. 40 - 60 mins.	$P < 0.001$	$P < 0.01$

injected was 0. 3 mg/kg bodyweight, and when this was altered the rates of loss were rechecked and it was found that similar significant differences in slope according to the period of estimation were exhib-:ited. To check whether pregnancy had any effect on the rates of loss of dye the results from 11 determinations made on sows during the first two weeks of pregnancy and 11 determinations, made in sows during the last two weeks of pregnancy were examined. No significant difference was found in either group from the previous pattern of results.

This curvilinear decline in the logarithm of the concentration of Evans blue in plasma with time indicates that the back-extrapolation of the mean slope during the period 10 - 60 minutes after injection will result in an underestimate of the concentration of dye in the plasma at zero time with a consequent overestimate of plasma volume. This overestimate could be as high as high as 5 - 10% if, for example, the rate of loss during the period 10 - 20 minutes represented the rate of loss during the mixing phase.

The cause of this deviation, in the pig, from the accepted constant rate of loss of the dye from the plasma in most other species, is not clear. There are reports in the literature of a 'violet impurity' in commercial preparations of Evans blue (Allen and Orahovats 1950). This impurity is thought to be lost very rapidly from the bloodstream whereas none is lost from the standard. During the experiments reported, however, only one batch of dye was used throughout. This was tested for the presence of the violet impurity using paper chromo-:tography with water as the mobile phase, and no impurity was found. The relevance of the violet impurity may be of doubtful importance as Allen and Orahovats (1950) noted that its peak light absorption wave-:length was 500 mμ. Thus, as the standard solution is measured at 620 mμ, the presence of small quantities of the impurity would contribute little to the optical density of the standard solution.

It/

It was suggested that the amount of dye injected, 20 - 50 mg per animal, was too great for the available sites on the plasma albumin and that some dye was not bound to the albumin. This free dye would then be lost from the plasma more rapidly than the bound dye. This however is unlikely on theoretical grounds, since Rawson (1943) estimated that there were 8 - 14 sites for Evans blue on the plasma albumin in vitro while Allen and Orahovats estimated that there were 11 sites, again in vitro. It was estimated that, in the 21 determinations previously mentioned, the average plasma volume was 7,000 ml in adult sows. The concentration of albumin in pigs' plasma is approx-:imately 3.9% (Miller, Ullrey, Ackerman Schmidt, Hoefer and Luecke 1961). Therefore the total albumin in 7,000 ml plasma is 273 g. The molecular weight of albumin is 70,000 and that of Evans blue, 1,000 (Rawson 1943). Therefore to attach only one molecule of Evans blue to each albumin molecule $273 \times \frac{1}{70} = 3.99$ g. Evans blue would be required. At the time these determinations were made the average dose of Evans blue was 40 mg. and hence the ratio of labelled to un-:labelled molecules of albumin was $\frac{0.04}{3.99} \simeq 1\%$.

The reason for the curvilinear relationship between the logarithm of concentration and time may possibly be linked with the fate of the dye-albumin complex. Dye, still firmly bound to the albumin, leaves the vascular system through the capillary walls; from the intersitial space it enters the lympatic system. Some dye is removed by phago-:cytosis in the reticulo-endothelial systems (Gibson and Evans 1937). However, in all species so far examined, dye has been found in the lymph of the thoracic duct soon after injection, (Courtice 1943; Barnes, Loutit and Reeve 1949). Courtice found Evans blue in the returning lymph within 15 minutes of injection in the dog and in the goat, and estimated that in dogs 0.59% and in goats 0.43% of the injected Evans blue had passed out of the thoracic lymph duct in one hour.

Barnes, /

Barnes, Loutit and Reeve (1949) estimated that in the dog 2% of the injected dye had returned to the circulation within one hour. The important comparison, however, is with the amount of dye lost from the circulation within one hour, which in the dog and goat is about 10 - 15%. (Courtice 1943).

It is possible that dye is lost from the circulation at a constant rate, but soon after injection this dye begins to return to the circula-:tion via the lymph ducts, thereby causing the deviations from a first order rate reaction already noted.

As time was not available to investigate this possibility, and as the estimations were all comparative and the error incurred slight, it was felt that until further evidence became available no confidence could be placed in any adjustments to the accepted method of inter-:preting the loss of Evans blue from plasma.

Section III The estimation of total blood volume and red cell volume from plasma volume and haematocrit in the pig.

a) The pattern of variation in the jugular haematocrit in untrained sows. The estimation of total blood volume from plasma volume depends upon the haematocrit. Since the haematocrit can vary very quickly under the influence of the spleen in certain species, and it was not know whether this phenomenon occurred in the pig, a study of the haematocrit was made.

Thirty-three Large white sows in various parities each had a catheter established in the jugular vein during the first two weeks of pregnancy. The animals were then housed in metabolism crates for three days during which tests were carried out. No further restraint of any kind was used. On the third day plasma volume was determined. The animals were quite untrained, in so far as none had been used previously in experiments requiring blood samples. Some of the animals had been housed in metabolism crates previously.

During/

During the determinations of plasma volume, blood samples were taken before the injection of Evans blue (0 minutes) and at 10, 20, 30, 40, 50 and 60 minutes after the injection of dye. Haematocrit determinations, using standard Wintrobe tubes spun at 3,000 x 'g' for 30 minutes, were made on the 0, 30 and 60 minute samples on the same day.

The results of this experiment are shown in Table 6. The variability in the haematocrit was almost 50% greater in the 0 minute sample than in the 30 or 60 minute haematocrits. This difference was statistically significant ($P < 0.01$). There was a significant difference between the haematocrit of the 0 - minute sample and the mean of the 30 and 60 - minute samples, the difference being signifi-:cant ($P < 0.001$). There was a small difference between the haemato-:crit values of the 30 - minute and 60 - minute samples but the variability in those samples was much less, so that the difference was still signifi-:cant ($P < 0.05$).

b) <u>The pattern of variation in jugular haematocrit in sows during train-:ing</u>. This experiment was carried out to determine whether the patterns of variation in jugular haematocrit shown by untrained sows was main-:tained by sows as they became used to the procedures involved in measuring blood volume. Turner and Hodgetts (1959) suggested that the very unusual 'F' cells correction of 1.22 which they derived from three very tame sheep was related to training.

The animals used in this experiment, 3 gilts, were housed in metabolism crates throughout pregnancy and were used in the detailed study of plasma volume changes in pregnancy described in Chapter 5, Section II. Catheters were established in the jugular vein within three days of service in all three gilts. In one animal the same catheter was used throughout pregnancy while a new indwelling catheter was estab-:lished in the opposite jugular vein during the tenth week in one animal and/

TABLE 6. **The change in jugular haematocrit with time after the beginning of the experiment.**

(observations on 33 adult sows)

Sampling Time min.	Mean Haematocrit and S. E.	S. D.
0	35.7 ± 0.63	± 3.63
30	31.9 ± 0.38	± 2.21
60	31.3 ± 0.43	± 2.46
Difference O.v. (30, 60)	4.04 ± 0.46	± 2.64 P<0.001
30 v. 60	0.57 ± 0.24	± 1.36 P<0.05

and during the twelfth week in the other. This was done because the original catheters were no longer patent.

Plasma volume determinations were made at frequent intervals and haematocrit determinations made on the 0, 30 and 60 minute samples.

The results of this experiment are presented in Table 7 and indicate that while the haematocrit of the 0 - minute sample showed a steady decline during pregnancy ($P < 0.001$) the mean haematocrit of the 30 and 60 minute samples showed no tendency to drop during pregnancy. The difference between the haematocrit of the 0 minute sample and the mean haematocrit of 30 and 60 minute samples also shows a steady decline during pregnancy ($P < 0.001$) which equals, almost exactly, the rate of decline of the haematocrit value in the first sample.

c) Discussion of subsections a) and b). The variations in jugular haematocrit during the measurement of blood volume in pigs can be explained if it is assumed that in the pig the spleen acts as a reservoir for red blood cells. The results in subsection a) would then indicate that when the animal was disturbed initially, either by the presence of a person, or by the removal of the bandages securing the end of the catheter, excitement was caused and the spleen contracted. This in turn liberated red cell rich blood into the general circulation raising the jugular haematocrit. As the determination of plasma volume pro-:gressed and the animal became less agitated by the procedures, the spleen dilated and the jugular haematocrit was lowered.

This situation is very similar to that described by Turner and Hodgetts (1959) in normal sheep. A catheter was established in the jugular vein of a sheep with sufficient length outside the sheep to enable an operator to sample blood from the sheep while in an adjoining room to the sheep. A blood sample was taken before and immediately after

a/

TABLE 7. The pattern of fluctuations in jugular haematocrit during plasma volume determinations, with time and pregnancy in 3 gilts.

Sow No.	Estimated weekly change in jugular haematocrit	
	1st Sample	1st. - (mean of 2nd. and 3rd)
861	-0.51 ± 0.13 P< 0.01	-0.58 ± 0.19 P< 0.05
862	-0.91 ± 0.21 P< 0.01	-0.88 ± 0.26 P< 0.05
863	-0.21 ± 0.28 N.S.	-0.34 ± 0.36 N.S.
Mean	-0.59 ± 0.13 P< 0.001	-0.64 ± 0.16 P< 0.001

a second operator entered the room containing the sheep. The haematocrit of the blood sample taken after the operator entered the room was considerably higher than the haematocrit of the sample taken before the operator entered. When the operator had been present in the room for 15 minutes, a further sample of blood was taken and the haematocrit was found to have dropped again. Splenectomised sheep, however, showed no such variations in jugular haematocrit.

The progressive decline in the haematocrit of the 0 - minute sample as pregnancy proceeded would indicate that as the animals became used to the procedures involved in the measurement of plasma volume, the initial excitement caused became progressively less and consequently the initial rise in haematocrit became less. This would, over the period of training, result in a progressively smaller decline in jugular haematocrit during blood sampling.

This suggestion is to some extent borne out by the behaviour of the pigs during the experiment. Initially the animals were very difficult to handle, they would not stand still, flapped their ears and occasionally snapped. As the experiment progressed the animals became very tame, lying down when scratched and allowing irritating procedures, such as the removal and replacement of adhesive bandages, to be carried out without protest.

The possibility cannot be ignored, however, that the steady decline in fluctuations in jugular haematocrit were due, at least in part, to pregnancy, in that owing to increasing plasma volume, the spleen withheld a progressively smaller proportion of the red cells from the circulation in order to maintain large vessel haematocrit. Thus when the animal was disturbed initially, the spleen still contracted to the same degree throughout pregnancy, but as pregnancy advanced the volume of red cells present in the spleen in the undisturbed animal decreased/

decreased. When the spleen contracted, the volume of red cells liberated into the general circulation was smaller and the jugular haematocrit was less elevated. However, the haematocrit fell steadily throughout pregnancy while the plasma volume, shown on figure 5, decreased initially and increased only during the last eight weeks of pregnancy.

From the evidence presented it is reasonable to presume that the spleen in the pig, as in the dog and the sheep "acts as a dynamic reservoir in which red cells in excess of oxygen capacity demanded by the existing situation are withheld from the circulation" (Turner & Hodgetts, 1959). The evidence also indicates that the volume of red cells withheld by the spleen can vary very rapidly under the influence of psychic stimuli and that pigs can become used to stimuli with a corresponding decrease in the splenic activity.

d) Conclusions on the use of the jugular haematocrit in the estimation of total blood volume in pigs. The fluctuations in jugular haematocrit in the pig make a reliable estimate of total blood volume and red cell volume from plasma volume and haematocrit almost impossible. Any determined 'F' cells correction for use in normal unanaesthetised pigs will be highly variable owing to excitement during the determination of the correction. The same will be true of an 'F' cells correction for use in anaesthetised pigs since there are degrees of depth of anaesthesia. Furthermore, the determination of red cell volume in unaesthetised animals in which the spleen acts as a reservoir for red blood cells is difficult. It was shown by Kraintz, De Boer, Smith and Huggins (1958) that mixing of a red cell label throughout the splenic red cells in anaesthetised dogs was not complete in 30 minutes. If the red cell volume was based on the activity (Cr^{51}) of a blood sample taken 10 minutes/

10 minutes after the injection of the label, the total red cell volume was underestimated by 11.3% $\pm$ 3.15. Lawson, Shanklin and Kabal (1959) estimated that complete mixing of label (P^{32}) in the spleen in dogs required at least 40 minutes and that errors due to incomplete mixing could be as high as 25%.

The only 'F' cells reported in pigs (Talbot & Swensen, 1963); is numerically very low, 0.71, and has such a large standard deviation, $\pm$ 0.08, as to severely limit its usefulness. No details were given as to whether it was obtained under anaesthesia or not, but its magnitude might be explained either by suggesting that if it was determined without anaesthetic the jugular haematocrit was greatly elevated, or if it was determined under anaesthesia, the red cell volume was underestimated. Talbot and Swensen determined plasma volume using Evans blue and if, as suggested in this work, this method overestimates plasma volume, in the pig, a further error may have been incurred.

The most likely solution to the problem of determining an 'F' cells correction in the pig is to determine the red cell volume in unanaesthetised animals given adrenalin intravenously. This would contract the spleen and ensure complete mixing of the label. Adrenalin would then be used at every determination in which this 'F' cells was used and would ensure a uniformly contracted spleen.

So complex were the problems involved in determining a useful 'F' cells correction in the pig, that it was decided that such a determination was beyond the scope of the present series of experiments.

However, it was desirable to make some estimate of total blood volume and red cell volume in the present series of experiments. Since the haematocrit determined from samples taken 30 - 60 minutes after the injection of Evans blue was less variable and the animals appeared to be less excited at this time, it was decided to use the mean of the 30 and 60 - minute haematocrits to calculate total blood volume from plasma volume. In this way errors due to excitement would be reduced/

reduced and a useful estimate of total blood volume, at least on a comparative basis, could be obtained.

If, however, as pregnancy advances the spleen in the unexcited pig contains a progressively smaller proportion of red cells, the relationship between the jugular haematocrit and true body haemato-:crit will alter. It is possible that the use of the mean of the 30 and 60 minute haematocrits to estimate total blood volume, will make estimates of total blood volume comparable between animals at any one stage of pregnancy but not within animals over the period of pregnancy.

Section IV General conclusions on the methods used to measure plasma volume, total blood volume and haematocrit in the present experiments.

1) The use of the dye Evans blue to measure plasma volume in the adult pig gives a repeatable estimate plasma volume. The loss of Evans blue from the plasma is not a first order rate reaction and the accepted method of determining concentration of Evans blue in plasma at time O involves an underestimate of dye concentration. This in turn results in a slight but consistent overestimate of plasma volume. In this series of experiments absolute values are not of prime importance and comparative values suffice.

2) The estimation of total blood volume from plasma volume involves several further errors;

(a) the lack of a correction factor for trapped plasma will result in an overestimate of total blood volume and red cell volume. This is likely to be small and consistent.

(b) the lack of an 'F' cells correction will result in errors in the estimation of absolute values for total blood volume from plasma volume and haematocrit. The use of the 30 and 60 - minute haemato-:crit will reduce errors due to psychic stimulation of the pigs.

When this 'normal' haematocrit is used, it is possible that red cell/

cell volume is comparable only between animals at the same stage of pregnancy and not within animals over the course of pregnancy.

3) No accurate measurement of total blood volume in the pig over pregnancy can be made unless both plasma and red cell volume are measured simultaneously and adrenalin is used on all occasions to contract the spleen.

CHAPTER 4 STUDIES OF THE BLOOD VOLUME IN SOWS DURING FIRST TWO WEEKS OF PREGNANCY.

Section 1 The mean values of plasma, red cell and total blood volume and their relationship with the liveweight of the sow.

a) Introduction. The aim of this study was to determine the mean plasma, red cell and total blood volumes and the haematocrit and haemoglobin concentration in the blood of sows in the first two weeks of pregnancy. These mean values and the deviations from them would provide a basis for the study of these parameters in pregnancy and enable the significance of changes in them during pregnancy to be assessed. The relationships between plasma, red cell and total blood volume with liveweight were also studied since these parameters are associated in all species so far studied.

The first two weeks of pregnancy were chosen as the base line, since at this time the sows were not lactating, and it was considered that they would have recovered from possible upsets in fluid balance brought about by abrupt weaning. It was hoped that the effects of pregnancy would not have begun to effect the plasma or red cell volume at this stage.

b) The animals and their treatment. During the course of the experiments, which lasted 18 months, 34 sows were selected for use in the experiment. The group of sows was made up of 6 first parity, 2 second parity, 6 third parity, 13 fourth parity, 5 fifth parity and 2 sixth parity animals. The animals were all Large White sows drawn from the Institute herd, and they had a variety of nutritional histories. The animals which were in their first, second or third parities when the estimations were made had been given, during all their pregnancies, a standard level of 2.2kg/ day of diet R.S.I. (Appendix 1). During lactation they had been given a standard diet but at levels related to the number of their progeny. The animals which were at the beginning of their fourth, fifth and sixth parities when/

when they were used in the experiment had been given treatment identical with that given to the younger sows since the beginning of their fourth parity. During their first three parities, however, they had been used in a number of nutritional trials and had been given varying levels of a number of diets of widely different energy and protein contents, both during pregnancy and during lactation. This resulted in considerable variation in the liveweight of these sows. This variation was especially marked in the fourth-parity sows since they had been given the standard level of 2.2 kg feed per day for the shortest time.

During the first or second week of pregnancy a catheter was established in the jugular vein of each pig. The animal was weighed and then placed in a metabolism crate, where on the following morn-:ing a glucose tolerance test (see Chapter 7) was performed. On the following morning a determination of plasma volume was made and total blood volume and red cell volume estimated using the mean of the 30 and 60 - minute haematocrit. Haemoglobin was measured using the oxyhaemoglobin method of Nicholas (1951).

c) Results and the effect of parity on the relationship between blood volume and liveweight. The results of this experiment were exam-:ined on a parity basis since it became apparent that there were trends in the results associated with parity. The results from the two second parity sows were included with those of the third parity and the two sixth parity with those of the fifth, so that the sows were classified in groups large enough for satisfactory statistical analysis. In Table 8 mean values and standard deviations of plasma, red cell, and total blood volumes haematocrit and haemoglobin concentration in blood are shown by parities. The mean plasma volume was found to rise with increasing parity until the fourth parity but the results for parity groups 4 and 5 - 6 were identical. The same trend was apparent in the red cell volumes but possibly because of the inaccuracies of measurement/

TABLE 8. Mean values and standard deviations of some components of blood in sows in early pregnancy.

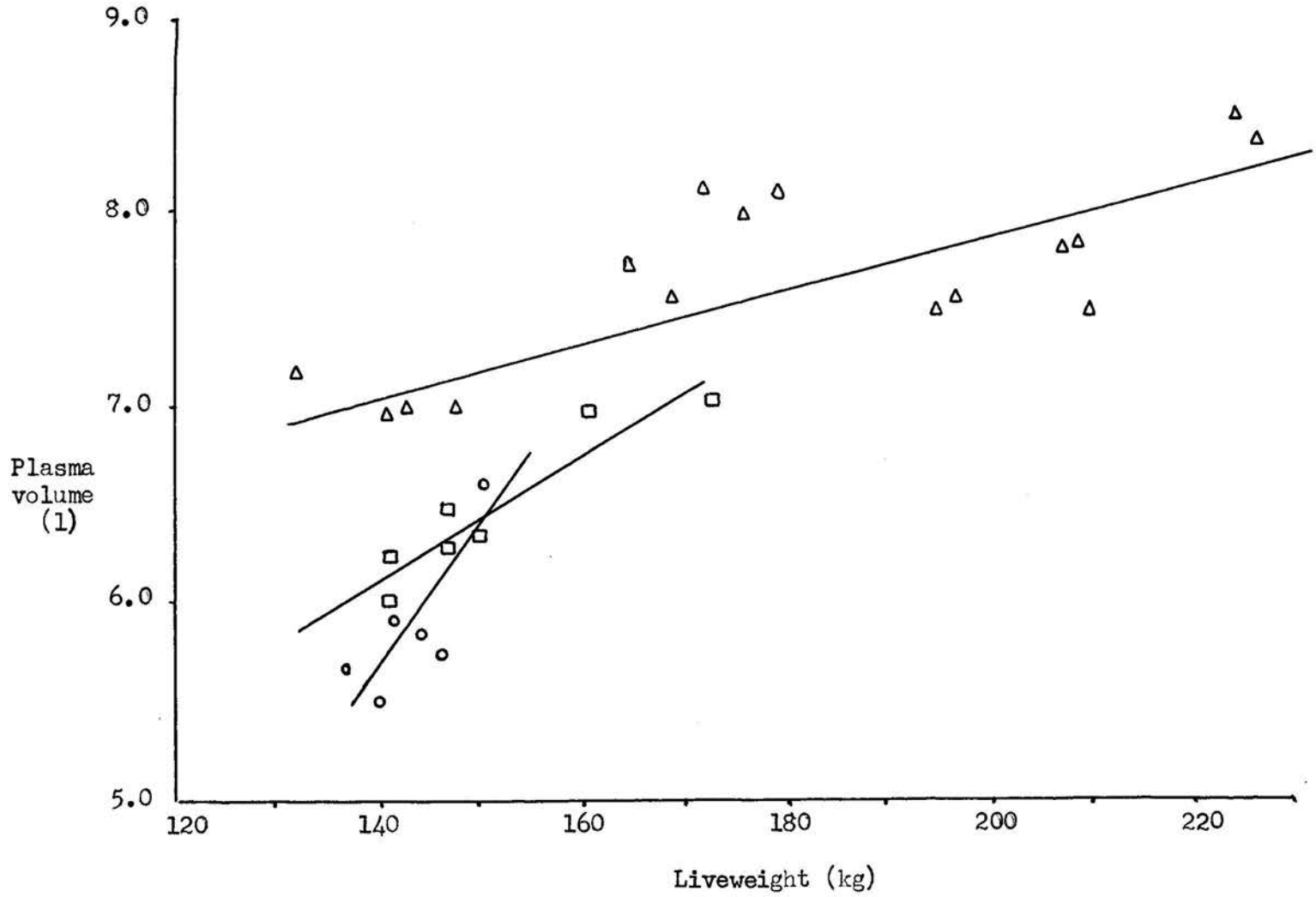
Measurement	Unit	Parity			
		1	2 - 3	4	5 - 6
No. of sows		6	8	13	7
Plasma volume	Litres	5.91 ± 0.37	6.50 ± 0.40	7.59 ± 0.55	7.63 ± 0.56
Red cell volume	Litres	2.83 ± 0.42	3.10 ± 0.45	3.43 ± 0.55	3.67 ± 0.49
Total blood volume	Litres	8.73 ± 0.75	9.60 ± 0.81	11.02 ± 1.01	11.29 ± 0.99
Haematocrit	%	32.2 ± 2.3	32.2 ± 2.2	31.0 ± 2.6	32.4 ± 2.0
Haemoglobin	g/100ml	11.0 ± 0.81	11.0 ± 1.05	11.0 ± 1.15	11.4 ± 0.72
Liveweight	kg.	144 ± 4.4	152 ± 11.0	183 ± 32.0	169 ± 17.0

FIGURE 4. The relationship between plasma volume and liveweight in sows during the first two weeks of pregnancy.

△ 4th., 5th., and 6th., parity sows.

□ 2nd and 3rd parity sows.

○ 1st parity sows.


9.0
8.0
7.0
6.0
5.0
Plasma volume (l)
120
140
160
180
200
220
Liveweight (kg)

measurement the trend is less clear, and the mean red cell volume is slightly higher in parity group 5 - 6 than in parity group 4. Within a parity group the standard deviation of the plasma volume was small, in the order of ± 6 - 7% of the mean. The red cell volume showed a greater deviation but this was to be expected with the method of determination used.

The haematocrit and haemoglobin concentration in the blood did not alter with parity and were extremely constant.

On analysis of the relationship between liveweight and plasma, red cell and total blood volumes it was found that a different relation-:ship could be derived for each of parity groups 1, 2- 3 and 4. How-:ever, it was found that the same expression described the relation-:ship between liveweight and plasma, red cell and total blood volumes in parity groups 4 and 5 - 6. The number of parity groups was, therefore, reduced to three, in this analysis.

In Table 9 the relationships between plasma volume (y_1), red cell volume (y_2) and total blood volume (y_3) and liveweight (x_1) are shown. Although the difference between the regression coefficients of plasma, red cell and total blood volume on liveweights failed to reach significance, there is some indication of a downward trend with increasing parity. The relationship between plasma volume and liveweight is displayed graphically in Figure 4.

With the exception of the first parity sows, a better estimate of red cell volume was obtained by taking haematocrit (x_2) into account as well as liveweight, Since the haematocrit value was used to estimate red cell volume, this result is not altogether surprising and a similar result was found for total blood volume. For the sake of completeness, the regression equations on liveweight and haematocrit have been given for plasma volume as well as red cell volume in the results shown in Table 10.

With/

TABLE 9. The relationships between blood volume and liveweight with parity in sows.

(x_1 = liveweight)

Parameter	Parity	Equation	R. S. D.
Plasma volume	1	$y_1 = 0.07x_1 - 4.21$	0.26
	2-3	$y_1 = 0.032x_1 + 1.62$	0.20
	4, 5-6	$y_1 = 0.013x_1 + 5.28$	0.40
Red cell volume	1	$y_2 = 0.09x_1 - 10.18$	0.14
	2-3	$y_2 = 0.026x_1 - 0.86$	0.38
	4, 5-6	$y_2 = 0.010x_1 + 1.73$	0.47
Total blood volume	1	$y_3 = 0.16x_1 - 14.39$	0.35
	2-3	$y_3 = 0.058x_1 + 0.76$	0.54
	4, 5-6	$y_3 = 0.023x_1 + 7.02$	0.77

TABLE 10. The relationships between blood volume, and liveweight and haematocrit with parity in sows.

Parameter	Regression Equation		R.S.D.
Plasma Volume	$y_1 = 0.014x_1 + 0.04x_2$	(+ 2.60 (Parity 1) (+ 3.08 (Parity 2-3) (+ 3.85 (Parity 4-6)	± 0.35
Red Cell Volume	$y_2 = 0.007x_1 + 0.17x_2$	(- 3.66 (Parity 1) (- 3.44 (Parity 2-3) (- 3.08 (Parity 4-6)	± 0.17
Total Blood Volume	$y_3 = 0.021x_1 + 0.21x_2$	(- 1.07 (Parity 1) (- 0.36 (Parity 2-3) (+ 0.76 (Parity 4-6)	± 0.51

x_1 = liveweight.

x_2 = haematocrit.

With the inclusion of a term based on haematocrit there was no real indication of any difference in the regression coefficients from parity to parity, but in all parameters the constant terms show an increase from parity 1 to parities 2 and 3 and from parities 2 and 3 to parities 4, 5 and 6.

d) Discussion. The results shown in the previous section indicate that the volumes of plasma and red cells present in a sow during the first two weeks of pregnancy are a function of the liveweight of the sow. They have also shown that the relationship between plasma red cells and liveweight is not constant but is a function of the parity of the sow.

These results are readily explained if it is assumed that plasma and red cell volumes are related to lean body mass rather than live-:weight (Gregersen and Rawson, 1959). The ratio between lean body mass and adipose tissue in the body will, therefore, affect the relationship between plasma and red cell volumes and the liveweight.

The ratio of lean body mass to adipose tissue is affected by a number of factors, one of the most important being the physiological age of the animal. The young, rapidly growing animal increases its lean body mass very rapidly but as the animal approaches maturity, each unit increase in liveweight contains a greater proportion of adipose tissue. Eventually, when the animal has reached maturity, the lean body mass will remain relatively constant and changes in liveweight, excluding alimentary contents in the non-pregnant animal, will be attributable largely to fat. Thus in any one breed or strain of pigs, the lean body mass in the mature animal will be relatively constant, and differences in liveweight between mature animals will be a reflection of the amount of fat in the animal's body. This is borne out by the fact that the mean and standard deviations of the plasma/

plasma volume in parity groups 4 and 5 to 6 is constant, while the mean and standard deviation of the liveweights are different. On this basis it may be assumed that the sows used in this study are fully mature at the beginning of their fourth parity.

The tendency of the regression coefficient to decrease with increasing parity in the equations relating plasma, red cell and total blood volumes with liveweight (Table 9) can also be explained in a similar manner. Increasing parity until the fourth could be con-:sidered as an index of increased maturity, and thus in the first parity group each unit increase in liveweight will contain a greater proportion of lean tissue, and be related with a greater increase in plasma and red cell volumes, than each unit increase in liveweight in parity group 2 to 3. The same situation will occur between parity groups 2 to 3 and 4, 5 and 6.

In the second group of equations in which haematocrit is included (Table 10), and a constant regression coefficient used for all parties in each paramenter, the significant increase in the constant term of the equation with parity can also be explained in terms of the fat to lean ratio in the body. Since the constant term increases with parity a mature sow will tend to have more plasma and red cells than a less mature sow of the same weight and haematocrit. However, a more mature sow will only be the same weight as a younger sow either because the more mature sow is very thin or because the less mature sow is very fat. In either case the more mature sow will have a greater lean body mass and hence higher plasma and red cell volumes than the younger sow.

The precision with which plasma volume and red cell volume can be estimated from liveweight using the various equations is also worthy of note. If plasma volume is estimated from liveweight alone (Table 9) the/

the residual standard deviations of the equations varies from 200 - 400 ml. When expressed as a percentage of the mean of the appro-:priate parity group this is a deviation of from 4 to 5%. This is a very precise estimate especially when it is noted that the standard deviation of repeat estimates of plasma volume using Evans blue was ± 2.5%.

When red cell volume is estimated from liveweight and haema-:tocrit, the residual standard deviation for all parity groups is ± 170 mls. This represents an error of from ± 4.8 to 6% when expressed as a percentage of the mean value. Again the repeat-:ability of the Evans blue determination of plasma volume must be borne in mind. Although this estimate is precise, it must be stressed that it is only an estimate of the already rather crudely estimated red cell volume.

From these equations it is possible to estimate plasma and red cell volumes accurately, but the use of these equations must be restricted to the class of animal from which they were derived. The equations relating to the immature animals, parity groups 1 and 2 to 3 can relate only to animals given the same nutritional treatment and within the same weight range, since a different daily energy or protein intake could give rise to a different ratio of fat to lean in the body at the same liveweight. On the other hand, animals which would be classified in the first parity group but which had a higher liveweight although given the same nutritional treatment as the present series might well be more mature. Indeed the plasma volume of the heaviest animal in parity group 1 could be estimated with equal precision using the equation relating plasma volume and liveweight in parity group 2 - 3 (Figure 4). It is difficult to use these equations when consider-:ing the significance of rises in plasma and red cell volume in pregnancy/

pregnancy in immature animals, since the weight gain in pregnancy increases the animals' liveweight so that it lies beyond the scope of the equations derived from its particular parity group in the early pregnant stage. The equations derived for the mature animals, parity groups 4, 5 and 6, are more useful in assessing the signifi-:cance of changes in blood volume during pregnancy, since the weight gain in pregnancy does not increase the animals' weight so that it lies outside the scope of the appropriate equation.

It must be stressed again that these equations relating plasma and red cell volumes to liveweight apply only in the conditions prevail-:ing within the pig herd studied. This is especially true of the imma-:ture animals but will also apply to the mature animals, if the mean lean body mass varies greatly between breed and even perhaps between strains of the same breed.

Section II A comparison of the estimates of plasma volume and red cell volume made in the present study with those reported by other authors.

a) The basis for comparison. In Chapter 2 it was pointed out that the estimate of plasma and red cell volume made in any one animal could vary according to the methods used. It is interesting therefore to compare the estimates made in this study with those made elsewhere by other authors.

There are difficulties, however, in making useful comparisons since almost all the reported estimates of blood volume in pigs have been made using immature, growing and fattening, pigs. In the previous Section it was shown that the relationship between plasma and red cell volume varied with maturity. Unfortunately, in the reported estimates of blood volume, there is no indication of the maturity of the animals measured, and only their liveweight is quoted. In order to make useful comparison it was decided to confine the comparisons to animals whose liveweights lay approximately in the range 100-150 kg, in/

in the hope that this would, to some extent, minimise differences in maturity. In order to minimise possible differences in body composition further, it was decided that the only figures from this study which would be considered were those from the first parity animals. The plasma and red cell volumes of these animals had been measured at the beginning of their first pregnancy and since they had undergone neither pregnancy nor lactation, they could be considered as heavy growing and fattening pigs.

b) The treatment of the material. It is intended to discuss the estimates of plasma and red cell volume reported in four separate papers; Bush, Jensen, Cartwright and Wintrobe (1955), Hansard, Sauberlich and Comar (1951), Doornenbal, Asdell and Wellington (1962) and Doornenbal and Martin (1965).

All these authors measured red cell volume and estimated the plasma volume from this and the jugular haematocrit. The mean plasma and red cell volumes, the mean plasma and red cell volumes per kilogram of liveweight, the liveweight and the reported jugular haematocrit are shown in Table 11.

The mean red cell volumes per kilogram of liveweight reported in this study are very similar, especially since liveweight alone is used as a basis for comparison. It was somewhat unexpected that the mean red cell volume per kilogram liveweight estimated in this study from plasma volume and haematocrit should agree so closely with the other estimates, which were all obtained by direct measurement of the red cell volume. The estimates of red cell volume made by Doornenbal and Hansard were made in the conscious animal but those made by Bush and his associates were made in anaesthetised animals. Since Bush and his colleagues, using cells labelled with P^{32}, based their estimate of red cell volume on the specific activity

of/

TABLE 11. The plasma and red cell volumes in pigs weighing between 100 kg and 160 kg.

Authors	Weight of pig (kg)	Red Cell Volume (ml)	Plasma Volume (ml)	Red Cells /kg (ml)	Plasma /kg (ml)	Jugular Haematocrit (%)
Present study	144	2830	5910	19.7	40.8	32.2
Bush et al (1955)	110	2321	3901	21.1	35.4	37.3
Hansard et al (1951)	156	2763	4431	18.0	28.4	38.4
Doornenbal et al (1962)	111	2308	3186	20.8	28.7	42.0
Doornenbal and Martin (1965)	100	2353	2547	23.5	25.5	48.0

of a sample of blood taken 10 - 15 minutes after the injection of the labelled cells, it is possible that they may have underestimated the red cell volume. Kraintz et al (1958) showed that mixing of labelled red cells was not complete in anaesthetised dogs after 30 minutes. It is impossible to suggest by how much Bush and his colleagues may have underestimated red cell volume since Kraintz et al (1958) showed that the underestimate declined from a mean of 11% to 3% between 10 and 20 minutes after mixing.

The estimates plasma volume / kg in the various studies vary very greatly, with that made in the present study (highest), and those made by Hansard and Doornenbal (lowest), while that made by Bush lies approximately mid-way between these estimates. On consideration of the methods used to determine plasma volume in the various studies it is possible to suggest reasons for the differences.

In their studies Bush and his colleagues also measured plasma volume using radio-active ferric chloride ($Fe^{59}Cl_3$) as a label. During the determinations the pigs were anaesthetised using 5% pentobarbitone, and it was noted, in a series of 50 determinations using normal pigs, that the haematocrit under anaesthesia was lower than the value before anaesthesia by amounts ranging from 0 - 13 ml red cells / 100 ml blood. The haematocrit under anaesthesia was used to estimate plasma volume from red cell volume and no correction for trapped plasma was made. When the estimates of plasma volume, made directly, using $Fe^{59}Cl_3$ as a label, were compared with those estimated from red cell volume and haematocrit, it was found that the directly-estimated plasma volume ($Fe^{59}Cl_3$) was on average 7% higher than the indirectly measured plasma volume, and that the range of deviations from this mean value was - 40% to + 50%. To explain these discrepancies Bush postulated that in using $Fe^{59}Cl_3$ as a label plasma volume was overestimated since "a propor-:tion of the iron leaves the circulation after injection". All the values for plasma volume quoted by Bush were, therefore, based on the red cell volume and haematocrit.

However/

However, if the trapped plasma in the red cell volume of the haematocrit tube was 5%, the plasma volume would be underestimated by approximately 8.0% as can be shown by the following calculation. In Table 11 the figures reported by Bush and his colleagues were; red cell volume 2321 ml, haematocrit 37.3 and plasma volume 3901 ml. If the haematocrit column contained 5% trapped plasma then the true haematocrit was 35.4%

$$\therefore \text{ True plasma volume} = \left(\frac{2321}{35.4} \times \frac{100}{1}\right) - 2321 \text{ ml}$$

$$= 4235 \text{ ml}$$

$$\therefore \text{ the error} = \frac{334}{4235} \times \frac{100}{1} = 7.9\%$$

Similarly if the red cell volume was underestimated, then the plasma volume would also be underestimated. It is possible that the large range of differences between the two estimates of plasma volume was due to the variations in the depression of haematocrit under anaesthesia. From the figures given by Bush et al it is possible to calculate that the mean depression of haematocrit under anaesthesia was 4.75 ml red cells / 100 ml blood. It is likely that degree of depression of haematocrit under anaesthesia is closely related to the state of excitement of the pig before anaesthesia and the depth of anaesthesia induced.

The effect of variations in haematocrit on estimates of plasma volume can be illustrated in the following manner using a "model pig" with a red cell volume of 2200 ml. If the haematocrit under anaesthesia is 35% then the plasma volume estimated from this will be 4086 ml. Assuming that this is an average pig, the plasma volume estimated using $Fe^{59}Cl_3$ will be 7% higher, i.e. 4372 ml, and the pre-anaesthesia haematocrit would be 4.75 ml red cells higher, i.e. 39.75%. Assuming that a number of determinations were made on the pig, and it showed the range of haematocrit depression under anaesthesia reported by Bush and his colleagues, the difference between the direct estimates

of/

of plasma volume and those made from red cell volume and haematocrit would range from - 23.7% to + 37.7% (Table 12).

There are, therefore, at least three sources of error in the estimate of plasma volume from red cell volume and haematocrit made under the conditions reported by Bush, and it is suggested that the direct estimate of plasma volume made using $Fe^{59}Cl_3$ as a label was the more accurate. The mean plasma volume reported by Bush could therefore be increased by 7% and the corrected value for pigs weighing 110 kg is shown in Table 13.

The estimates of plasma volume reported by Hansard et al (1951) were made from red cell volume and haematocrit determined in the conscious pig. The animals were restrained using a rope around the snout and blood samples obtained from the vena cava by the method of Carle and Dewhirst (1942); (Hansard, personal communication, 1966). It is suggested that the jugular haematocrit in these animals was considerably higher than normal because of excitement and that this resulted in a considerable underestimate of plasma volume (see Chapter 3).

Using the results from experiments in the present series it is possible to formulate an approximate correction factor to be used with the haematocrit of Hansard's animals. In the previous chapter it was noted that the mean haematocrit of 33 animals declined from an initial level of 35.7% $\pm$ 3.64% to 31.3% $\pm$ 2.46% (mean and S.D.) after 60 minutes (Table 6).

It is suggested, therefore, that the haematocrit values used by Hansard et al to calculate plasma volume should be lowered by at least the same proportion, $\frac{31.3}{35.7} = 0.87$. However, it is likely that the procedures/

TABLE 12. **The effect of various depressions of haematocrit under anaesthesia on the estimate of plasma volume from red cell volume and haematocrit.**

Depression	Haematocrit under anaesthesia (%)	Plasma Volume P^{32} (ml)	Difference P^{32} v Fe^{59} (%)
0	39.75	3335	- 23.7
2.5	37.25	3706	- 15.2
6.5	33.25	4417	+ 1.0
10.5	29.25	5321	+ 21.7
13.0	26.75	6024	+ 37.7

TABLE 13. Corrected values of plasma and red cell volumes in pigs weighing between 100 kg and 160 kg.

Authors	Weight of pig (kg)	Red Cell Volume (ml)	Plasma Volume (ml)	Red Cells/kg (ml)	PV/kg (ml)	Jugular Haema-:tocrit. (%)
Present study	144	2830	5910 ‡	19.7	40.8	32.2
Bush et al (1955)	110	2321 ‡	4158 *	21.1	37.8 *	37.3
Hansard et al (1951)	156	2763 ‡	5712 †	18.0	36.52 †	38.4
Doornenbal et al (1962)	111	2308 ‡	4153 †	20.8	37.4 †	42.0
Doornenbal et al (1965)	100	2353 ‡	3414 †	23.5	34.1 †	48.0

* recalculated using directly measured PV (Fe^{59})

† recalculated using 'F' cells of 0.85

‡ estimated

procedures used by Hansard caused greater excitement than those used in the present experiments. To allow for this extra excitement, it is suggested that the difference between the standard deviations of the haematocrit of the 0 - minute and 60 - minute samples in the present experiment, 1.2 ml red cells, (Table 6), be included in the suggested correction to Hansard's estimate of haematocrit. The correction factor now becomes $\frac{31.3}{(35.7+1.2)} = 0.85$.

On re-calculation using this correction factor, the mean plasma volume reported by Hansard, in the group of 4 pigs weighing 156.4 kg becomes 5,712 ml, which is equivalent to 36.5 ml plasma / kg liveweight.

Doornenbal et al (1962) also made their measurements in the conscious pig. The pig was restrained in a tight fitting crate and its head secured in a yoke. An ear was washed and shaved and the Cr^{51} labelled red cells injected through an ear vein. A blood sample was removed from the anterior vena cava 40 - 50 minutes later. These pro-:cedures also seem likely to cause an elevation of jugular haematocrit and it is suggested that the correction of 0.85 should be applied to the reported jugular haematocrit and the plasma volume re-calculated from the corrected haematocrit. The re-calculated plasma volume in the group of pigs weighing 111 kg is 4,153 ml, which represents a 37.4 ml plasma / kg liveweight.

Doornenbal and Martin (1965), using the methods reported by Doornenbal et al (1962), measured red cell volume and blood volume in a group of 8 pigs whose mean liveweight was 100 kg. In this group of pigs Doornenbal reports the average red cell volume as 2,353 ml and the average blood volume as 4,900 ml without comment. These figures represent a mean jugular haematocrit of 48% and a mean plasma volume of 2,547 ml or 25.5 ml plasma / kg liveweight. Even when the correction factor is applied the corrected haematocrit is still 40.8% and the plasma volume 3414 ml or 34.1 ml/kg. The mean

haematocrit/

haematocrit in the group, 48%, is very hard to explain. With sows which were very excited and struggling, haematocrits as high as 47% have been recorded in the present experiments but such high values are very rare indeed.

c) Conclusions, the corrected figures from different reports, for plasma and red cell volumes are summarised in Table 13 and show remarkable agreement with one another. Considering the possible errors in the method of estimating red cell volume from plasma volume used in the present experiment, the figures are remarkably similar. Although the correction to the plasma volumes reported by Hansard and Doornenbal make several assumptions it is of interest to note that these results agree so well with the corrected values of Bush et al, since these were corrected by a different method.

On the basis of this agreement of the estimates of plasma volume and red cell volume per kilogram of liveweight shown in Table 13, it is likely that the absolute values for plasma and red cell volumes quoted in this series of experiments are reasonably accurate estimates of the true plasma and red cell volumes occurring in the animals.

CHAPTER 5. STUDIES OF THE PATTERN OF CHANGE IN PLASMA VOLUME, RED CELL VOLUME, TOTAL BLOOD VOLUME, HAEMATOCRIT AND HAEMOGLOBIN CONCENTRATION DURING PREGNANCY AND LACTATION.

Section 1. Experiments on animals housed in the dry sow yard.

a) Introduction. The aim of this experiment was to investigate the changes, in a number of individual animals, of plasma, red cell, and total blood volumes, and haematocrit during pregnancy. Similar studies have been reported for humans, (Hytten and Leitch 1964) and for sheep (Barcroft, Kennedy and Mason 1939), but none have been reported for the pig. It was considered that information on the pattern of the changes in these parameters during pregnancy was essential in a study of their relationship with the birthweight of the piglets. Knowledge of these patterns would provide basic information on the physiology of pregnancy in the pig, and help to put measurements of these parameters made at isolated points during pregnancy into perspective.

This experiment was a pilot study, and was designed before methods of maintaining catheters for any length of time had been evolved. It was hoped that catheters could be inserted into the jugular vein on at least three separate occasions in each animal, and it was hoped to measure plasma volume, red cell volume and haematocrit during the second, ninth, and sixteenth weeks of pregnancy.

b) Materials and methods. Six large white sows were selected from the Institute herd. Five of the sows were litter sisters which had just completed their second lactation, while the sixth animal was a sow of the same age, but which had been non-pregnant for a period of approximately six months. All pigs were given 2.2 kg of a diet R.S.1. daily until they farrowed.

Approximately one week after service, each pig was removed from the rest of the herd, weighed and placed in a stout metabolism crate/

crate. A polyethylene catheter was inserted, and the animal confined to the crate for the entire period of the experiment, usually three or four days. On the day after the catheter had been inserted a glucose tolerance test was performed, and on the following day the plasma and red cell volumes were measured. After satisfactory estimates of glucose tolerance and plasma volume had been obtained, the catheter was removed and the animal returned to the dry sow yard.

During the ninth week of pregnancy this procedure was repeated and the sow returned to the dry sow yard. Approximately one week before farrowing the sows were again treated as before, but after the experiment they were moved into farrowing pens.

c) Results. The aim of this experiment, to plot the changes in plasma and blood volumes, was only partially achieved. This was due to the failure of the technique of inserting, and maintaining patent, the catheters. In mid-pregnancy, attempts to insert catheters were made in only five animals and success achieved in four instances. At the end of pregnancy all six animals were operated on, but catheters were established in only three animals. Of these animals, two had had catheters established in mid-pregnancy, while in the third no attempt had been made in mid-pregnancy, in order to preserve a suitable vein for the end of pregnancy.

The results which were obtained in this experiment are shown in Table 14. These results indicate that on average between the 10th and 66th day of pregnancy there was a slight increase in plasma volume although one animal showed no significant change. Over the same period red cell volume increased in two animals, did not change in the third and declined in the fourth, while the mean showed a slight rise. Between the 10th and 106th day both plasma and red cell volumes increased greatly in all three animals investigated. The general trend

suggested/

TABLE 14. Changes in plasma red cell volume and haematocrit in sows during pregnancy.

Pig No.	Determination 1				Determination 2				Determination 3			
	Days Pregnant.	Plasma Volume (ml)	Red Cell Volume (ml)	Haematocrit (%)	Days Pregnant	Plasma Volume (ml)	Red Cell Volume (ml)	Haematocrit (%)	Days Pregnant	Plasma Volume (ml)	Red Cell Volume (ml)	Haematocrit (%)
709	+18	6488	3053	32.0	-	-	-	-	-	-	-	-
711	+20	6375	2864	31.0	+63	7303	3597	33.0	-	-	-	-
715	+18	6604	2830	30.0	+64	7050	3094	30.5	104	8521	4010	32
756	+6	6986	3519	33.5	+74	7368	3467	32.0	108	9714	5004	34
713*	0	7022	3617	34.0	+64	6977	3283	32.0	-	-	-	-
710*	0	6301	2831	31.3	-	-	-	-	108	8763	3667	29.5
Average	10.3	6626	3127	32.0	66.3	7180	3360	31.9	106.7	9000	4227	31.8

* Animal returned to service.

suggested by these results is that between the beginning of pregnancy and the 66th day there is a slight rise in plasma volume and possibly in red cell volume, but from then until the 106th day both plasma and red cell volumes increase rapidly.

Throughout pregnancy there was no significant alteration in the haematocrit of the blood samples.

Section II. Experiments on animals confined to cages.

a) Introduction. The aim of this experiment was to overcome the difficulties in the use of catheters encountered in Experiment 1 and to determine more accurately the patterns of changes in plasmavolume red cell volume haematocrit and haemoglobin concentration in blood during pregnancy. It was also decided to investigate the changes in these parameters during lactation in the same animals, if possible.

The previous experiment showed that a catheter could be established in the jugular vein on only two occasions in any one pig. It was decided to try to overcome this disadvantage by maintaining individual catheters patent for long periods. Since pregnancy lasts for 114 days in the pig, each catheter would have to be maintained patent for at least 50. days to fulfil the requirements of the present experiment. When this experiment started no method of keeping catheters patent had been evolved and the methods described on pages 25-28 were developed during this experiment.

b) Treatment of the animals. Four non-pregnant gilts were selected from the Institute herd. The early management of these pigs, from eight weeks of age until service was as detailed by Elsley, Anderson, McDonald, McPherson and Smart (1966) During pregnancy the gilts were given 2.2 kg daily in two feeds of diet R.S.1. Water was given twice daily to appetite. During lactation the sows were given 1.8 kg daily of diet R.S.2. (Appendix 2) with a further allowance of 0.25 kg for each piglet suckled.

Three/

Three of the animals were served when they weighed just over 140 kg. Two days after the second service the animals were removed from the dry sow yard and a catheter established in the jugular vein. After each animal had recovered from the anaesthetic, it was placed in a stout metabolism crate where it remained, except for weighings, until the 110th day of pregnancy. The animals were removed to the farrowing house on that day and remained there until the piglets were weaned. The fourth animal was not served but received the same treatment otherwise and was used as a non-pregnant control.

The rooms in which the animals were kept were maintained at between 60° and 65° F.

Throughout pregnancy the catheters received constant attention and the details of the major manipulations are shown below.

Pig No. 861. A catheter was established on the day after service, (day 2) with difficulty, since only one vein was suitable. This catheter remained fully patent until the 56th day after service, and then ran sporadically until the 84th day, when a fresh catheter 3" longer was inserted through the same incission into the jugular vein. This catheter remained fully patent until the 156th day after service without further trouble.

Pig No. 862. This animal was the first of the three to be put on experiment and nearly all the techniques were developed on it. A catheter was established on the day after service and remained fully patent only until day 21. The catheter was patent sporadically between then and the 48th day when a fresh catheter was successfully inserted into the site of the previous one. This catheter was fully patent until the 84th day when it blocked. Attempts to insert a further catheter into the same site failed and a new catheter was inserted in the opposite jugular. This catheter functioned well until the 126th day after service when it became partially dislodged. A fresh/

fresh catheter was inserted into the same site and this remained fully patent until day 138., when the piglets completely dislodged it. This happened during the day, and when it was noticed the animal was immediately restrained and a fresh piece of catheter tubing inserted into the site of the dislodged one. This catheter remained patent for only a few hours, which was long enough for a determination of plasma volume to be made.

Pig No. 863. A catheter was established on day 2 and remained fully patent until day 50 and then sporadically until day 70. At this stage a mild infection set in and attempts to insert a new catheter into the same site failed. A new catheter was established in the opposite jugular vein and remained patent until the 110th day when it blocked completely. A fresh catheter was inserted into the same site but remained patent for only a few hours.

Pig No. 860, (non-pregnant) A catheter was established in the only suitable vein on the day after heat was observed. This blocked on the following day. From then until day 35 the catheter was patent only sporadically. On day 35 the catheter was dislodged and, after unsuccessful attempts to insert another catheter, the animal was discarded.

Throughout pregnancy determinations were carried out at 14-21 day intervals. During pregnancy these determinations were performed at 9.30 a.m., and during lactation at 2.30 p.m.

Total circulating haemoglobin was calculated from the mean con-:centration of haemoglobin in the 30 and 60 minute samples of blood and the total blood volume. All other determinations were made as previously described.

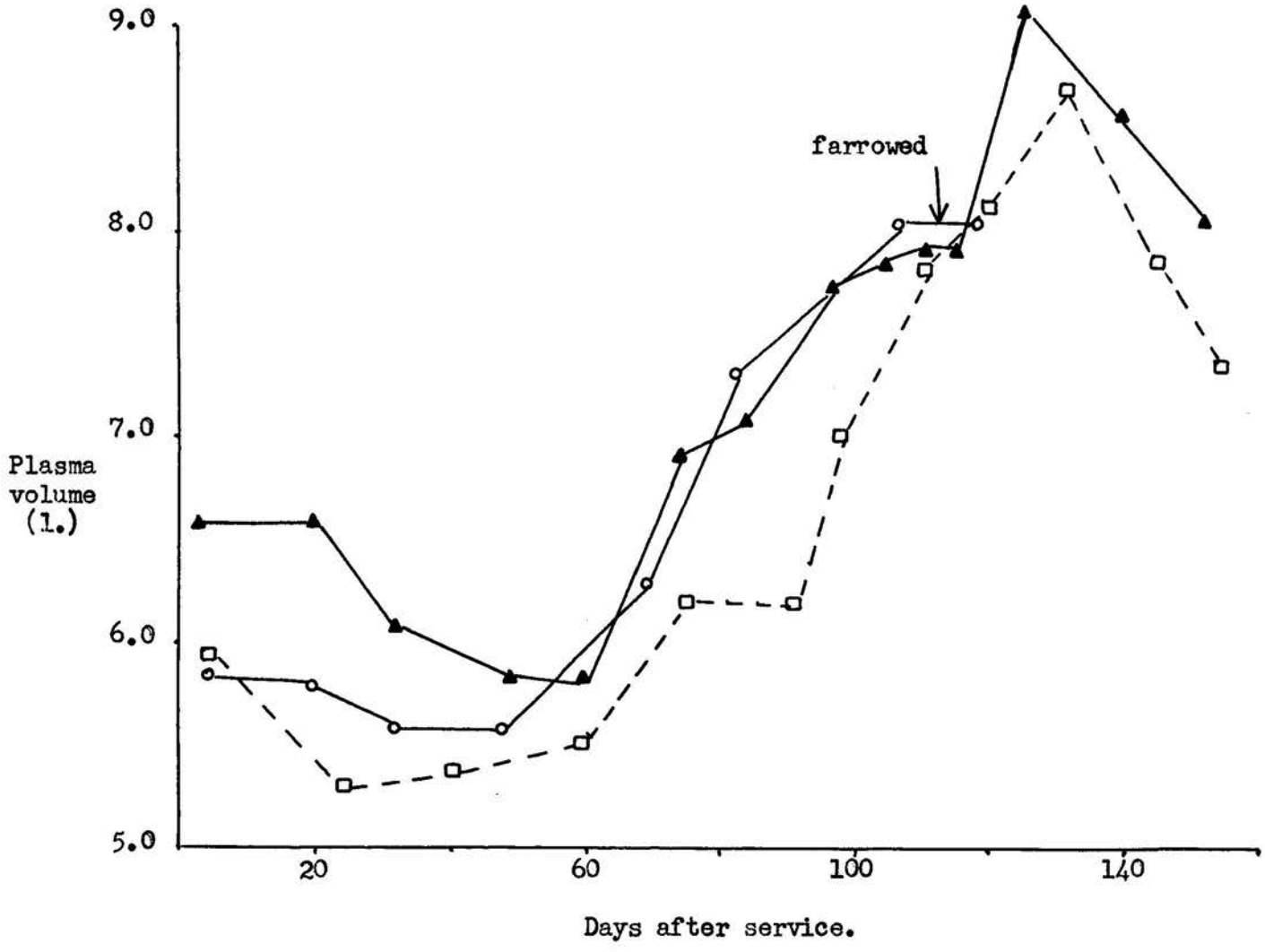
c) Results. The use of permanent indwelling catheters was more successful than anticipated. In the case of animals 861 and 862, the catheters were still patent after farrowing and the experiment was carried on/

FIGURE 5. Changes in plasma volume during pregnancy and lactation in gilts.

□-----□ pig No. 861.

▲——▲ pig No. 862.

o——o pig No. 863.


9.0
8.0
7.0
6.0
5.0
Plasma volume (l.)
farrowed
20
60
100
140
Days after service.

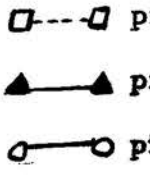


FIGURE 6. Changes in red cell volume during pregnancy and lactation in gilts.

□---□ pig No. 861.

▲——▲ pig No. 862.

○——○ pig No. 863.

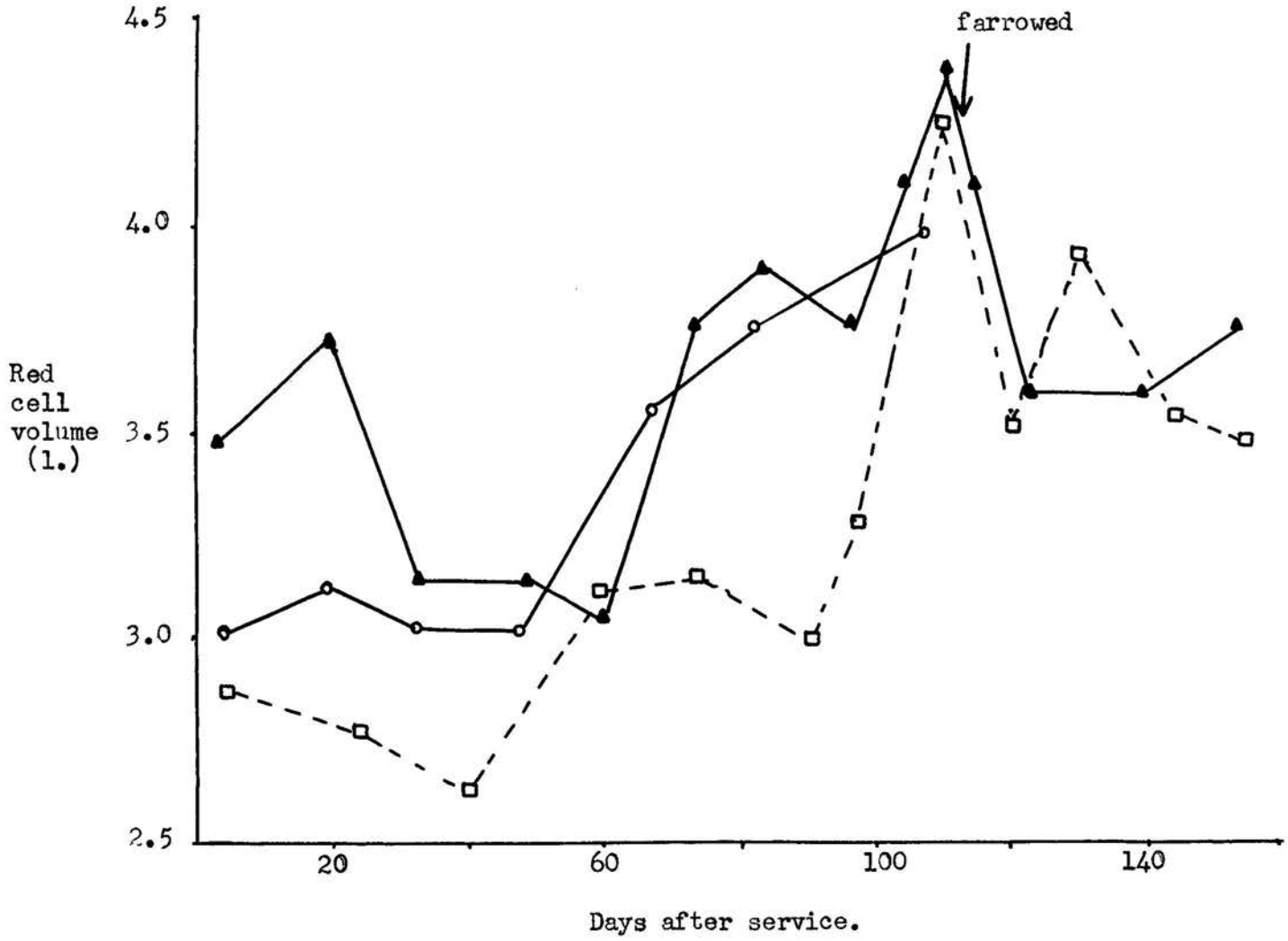

4.5
4.0
3.5
3.0
2.5
Red cell volume (l.)
farrowed
20
60
100
140
Days after service.

FIGURE 7. Changes in total blood volume during pregnancy and lactation in gilts.

□----□ pig No. 861.

△——△ pig No. 862.

○——○ pig No. 863.

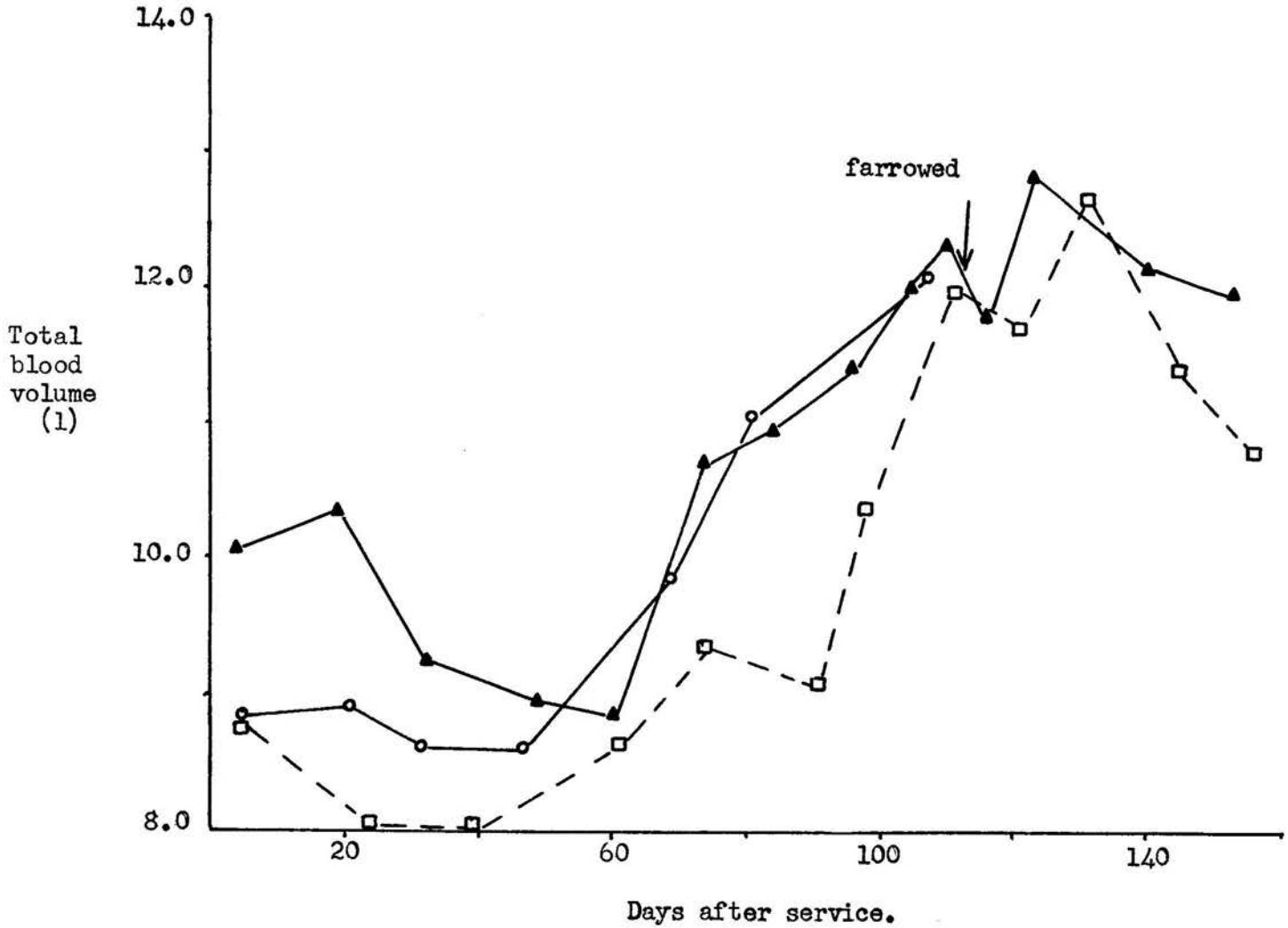
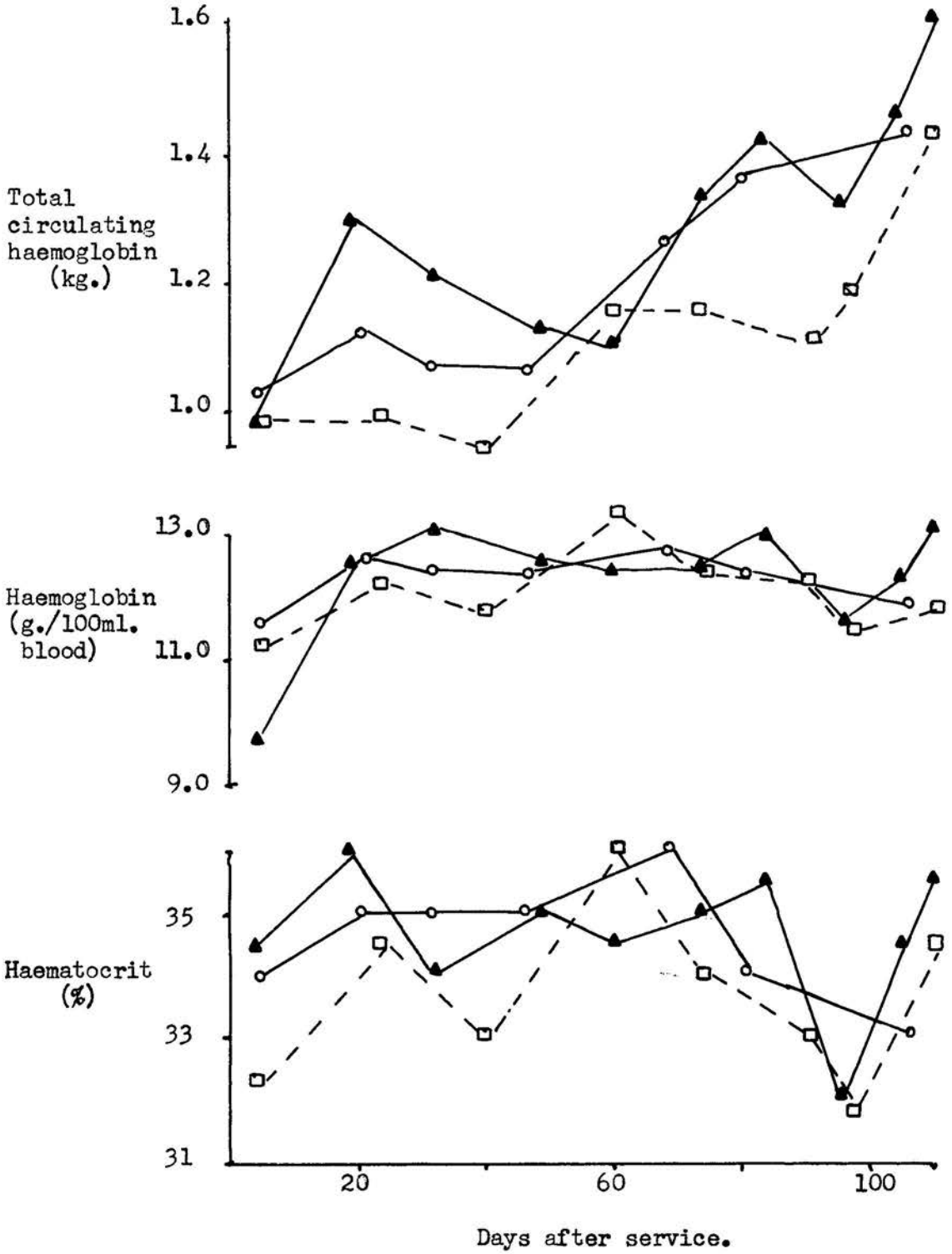

14.0
12.0
10.0
8.0
Total blood volume (l)
farrowed
20
60
100
140
Days after service.

FIGURE 8. Changes in haematocrit, haemoglobin concentration, and total circulating haemoglobin, in blood during pregnancy in gilts.

□----□ pig No. 861.

Δ——Δ pig No. 862.

o——o pig No. 863.


1.6
1.4
1.2
1.0
Total circulating haemoglobin (kg.)
13.0
11.0
9.0
Haemoglobin (g./100ml. blood)
35
33
31
Haematocrit (%)
20
60
100
Days after service.

on into lactation. It is possible however, that the results of the plasma volume determinations during pregnancy may not be completely comparable with those obtained during lactation since no study of the behaviour of Evans blue in vivo during lactation was made. For example the pattern of rate of loss may alter, though no evidence of this was noticed.

It was unfortunate, however, that no non-pregnant control values were obtained. An attempt to establish a catheter in a further non-pregnant animal ended in failure after seven weeks. Not only did the catheters run only sporadically for the entire period, but those estimates of plasma volume which were obtained showed considerable fluctuations which could not be checked. When this animal was discarded no further suitable animals were available because of a reduction in the size of the Institute herd prior to the establishment of a minimal disease herd.

The pattern of changes during pregnancy and lactation in the plasma volume, red cell volume, total blood volume, haemoglobin concentration and haematocrit for each animal are shown in figures 5, 6, 7 and 8.

The data obtained from the three gilts during pregnancy was analysed statistically, using orthogonal polynomials, for the existence of linear, cubic or quadratic trends during pregnancy. The individual measurements were made at irregular intervals during pregnancy because of difficulties with catheters, and in order to unify the data, estimates were made of the measurements at 0, 20, 40, 60, 80, 100 and 110 days after mating for each gilt, either by taking values within a few days of these times and assuming that there had been no change in the values, or by interpolating between two values, one on either side. The 110 - day value was excluded from the statistical analysis because some of the measurements were obviously changing very rapidly near farrowing, not necessarily in accordance with previous trends, and the exact/

exact timing of measurements was more important at this stage. The results obtained during lactation were not analysed statistically.

No attempt was made to formulate a regression equation to describe the pattern of change in plasma, red cell or total blood volume during pregnancy in all three animals since, although there were no significant differences in the trends shown by individual animals, the actual values were very obviously different. The results of the statistical analysis are shown in Table 15. Significant trends were found in all parameters except haematocrit, and trends did not differ significantly between animals.

The plasma volume in all animals declined after mating, reaching minimum values between 28 and 56 days after mating. Thereafter the plasma volume increased steadily returning to the mating levels at about 60 days after service and increasing steadily until farrowing on the 113th day. This quadratic trend was significant ($P < 0.001$). In both cases in which measurements were continued during lactation plasma volume continued to increase during lactation. In one animal No. 862, the increase in plasma volume appeared to stop over farrowing and then rise very steeply, while the increase in the other animal No. 861, did not appear to halt at all, but to continue to rise at the same rate over parturition. The peak plasma volume appeared to occur between the 14th and 21st days of lactation but since the plasma was changing so rapidly at this point, the peak in both animals was almost certainly missed. After reaching the peak, the plasma volume declined rapidly in both animals until weaning after 42 days of lactation. At weaning the plasma volume in No. 862 was the same as it had been immediately before parturition, while in No. 861 it was 400 ml below the parturition level.

Red cell volume, which was calculated from the plasma volume and haematocrit, not surprisingly tended to follow the pattern of the

plasma/

TABLE 15. The pattern of change of some components of blood during pregnancy in 3 gilts.

Measurement	Live-weight (kg)	Plasma Volume (l)	Red Cell Volume (l)	Total Blood Volume (l)	Hb. conc. (g/100ml)	Total Hb. (kg)	Haematocrit (%)
Mean Values:							
Day 0	147	6.1	3.1	9.3	10.9	1.00	33
20	145	5.9	3.2	9.1	12.5	1.14	35
40	149	5.6	2.9	8.6	12.4	1.06	34
60	167	5.9	3.3	9.2	12.9	1.18	35
80	180	6.8	3.6	10.4	12.5	1.31	34
100	187	7.6	3.7	11.3	11.8	1.31	33
110	193	8.0	4.2	12.1	12.4	1.50	34
Trend over	Cubic	Quadratic	Linear	Quadratic	Quadratic	Linear	None
Days 0 - 100	***	***	**	***	**	***	
Residual S.D. (after fitting trend)	± 4.6	± 0.33	± 0.24	± 0.56	± 0.58	± 0.094	± 1.1

plasma volume, but the dip to minimum values after service was not quite significant. ($0.05<P<0.1$) and the change in red cell volume in the period 0 - 100 days had a greater tendency towards a linear rise. ($P<0.01$). The mean red cell volume of the three animals shows a considerable rise between 100 and 110 days but when the individual values were considered this became less apparent (Figure 6.) During lactation the results of estimates of red cell volume may have been less reliable than those made during pregnancy, since the presence of the piglets made the sow less quiet and more easily annoyed by human presence. The uneasiness shown by the sow was greatest when the piglets wished to suckle her. However, in both animals there was a marked tendency for the red cell volume to decline immediately after parturition until about the 7th day of lactation. Thereafter the estimated red cell volume fluctuated. In pig No. 861 the estimated red cell volume between the 8th and 43rd days of lactation was constant with the exception of the estimate made on the 18th day. No explanation of this high value can be given, but it could be due to excitement, especially since the deviation from the value obtained on the other three occasions could be accounted for if the haematocrit were lowered by only 2 ml red cells per 100 ml blood. After the 7th day of lactation the estimates of red cell volume in animal No. 862 were constant except for the value obtained on the 38th day of lactation. However it should be pointed out that on that day a fresh catheter had been inserted, and the sow had been removed from her litter for two or three hours. The animal was excited and the deviation of the red cell volume obtained on this day from that obtained on the 10th and 27th days of lactation could be accounted for by a drop in haematocrit of only 1.5 ml red cells per 100 ml blood. The best descrip-:tion of the pattern of change in red cell volume during lactation, from the available material, appears to be that it dropped until the 7th day of lactation and then remained relatively constant.

The/

The total blood volume (Figure 7), the sum of the red cell and plasma volumes, followed the trend of the plasma volume during pregnancy - an initial dip followed in the second half by a steady increase. This quadratic trend was significant ($P < 0.001$). During the first 8 days of lactation while the plasma volume rose, the red cell volume declined, and the total blood volume showed a net decrease in both animals. Total blood volume then increased again in response to the increasing plasma volume, reaching a maximum on the 10th day in No. 862 and on the 18th day in No. 861. Thereafter total blood volume decreased until the end of lactation, following the pattern of the plasma volume.

The haemoglobin concentration in blood during pregnancy increased sharply during the first 20 days to a plateau, and then showed a tendency to fall away again between the 80th and 100th days (Figure 8). This quadratic trend was significant, ($P < 0.01$).

The total circulating haemoglobin rose from 1000 g. at the beginning of pregnancy to 1300 g. on the 100th day. This rise was best described as linear ($P < 0.001$) (Figure 8).

The details of the pattern of changes in haematocrit during this experiment have been described fully in Chapter 3, Section IIIb. However, the mean haematocrit of the 30 and 60 - minute samples showed no significant change during pregnancy. There is, however, an apparent tendency for the haematocrit to decline between the 80th and 100th days of pregnancy and to increase between the 100th and 110th days (Figure 8).

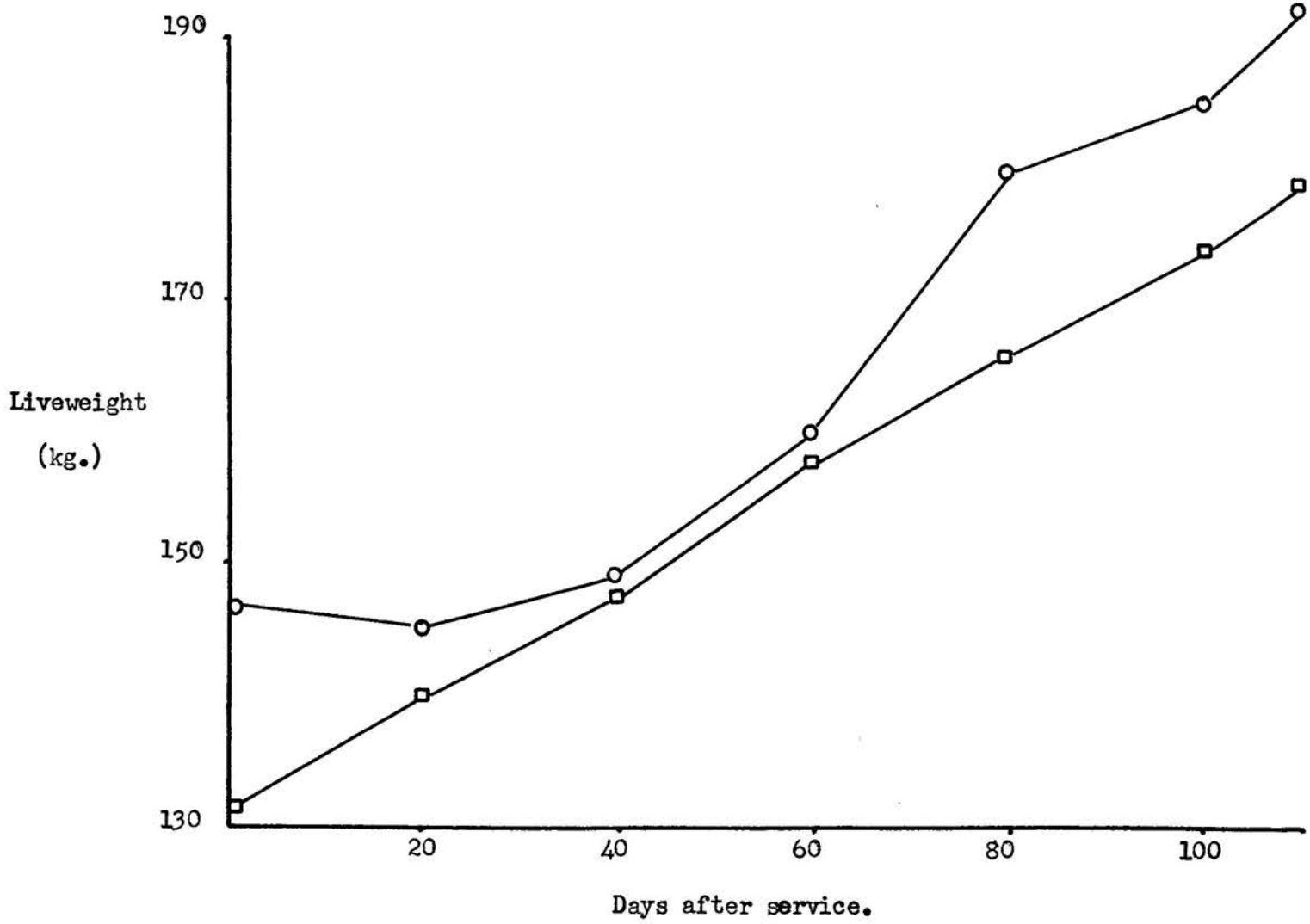
The liveweight of the gilts did not increase during the period 0 - 40 days, then increased rapidly between the 40th and 80th days and then less rapidly until the 100th day. This cubic trend was significant ($P < 0.001$).

Section III/

FIGURE 9. Liveweight changes during pregnancy shown by two groups of gilts given identical feed, but either restricted to metabolism crates or housed in the dry sow yard, throughout pregnancy.

o——o housed in metabolism crates (n=3)

□——□ housed in the dry sow yard (n=10)


190
170
150
130
Liveweight
(kg.)
20
40
60
80
100
Days after service.

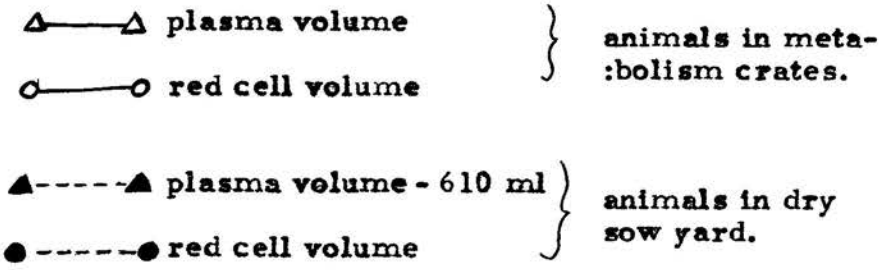
Section III. Discussion.

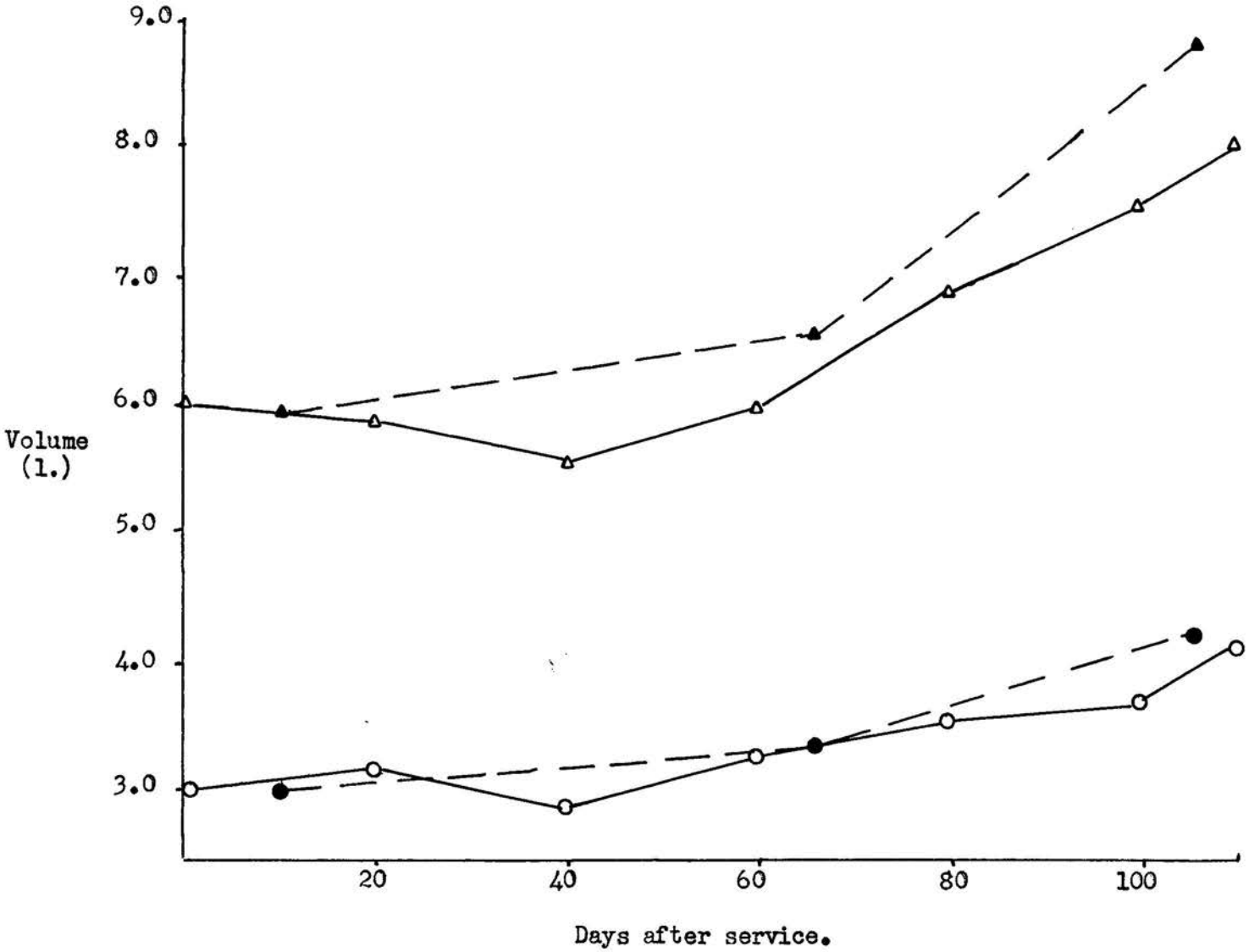
a) The effect of confinement. The results of the experiments described in the two previous sections indicated definite trends in the blood components during pregnancy. However since the results were obtained largely from Section II, in which the animals were confined throughout pregnancy, it is of importance to consider whether this confinement affected the results in any way. This will be done by comparing the results obtained in Section II with those obtained from either Section I or from groups of similar animals.

The pattern of liveweight changes during pregnancy of the animals restricted to crates is quite different to that shown by a group of 9 gilts reared along the same growth curve to service and given the same daily ration of the same diet throughout pregnancy, but housed during pregnancy in the dry sow yard. A comparison of the liveweight changes during pregnancy of these two groups is shown in Figure 9. The gilts housed in the dry sow yard received regular exercise and showed a steady linear increase in weight, while those animals confined to crates showed the cubic growth curve mentioned previously. The two groups are not altogether comparable, the free gilts being pregnant in the period June to September 1965, while the gilts housed in metabolism crates were pregnant between November 1965 and March 1966. Furthermore the gilts restricted to metabolism crates were served at a higher average weight, 147 kg., than the free gilts, 132 kg. It seems likely, however, that at least part of the different pattern in liveweight change was due to the animals being housed in metabolism crates through pregnancy.

It is not possible to say with any certainty whether the abnormal pattern of liveweight changes reflects abnormalities in the patterns of change in plasma red cell and total blood volumes. The reduction in plasma volume to minimum values between 28 and 56 days of the animals confined to metabolism crates could be at least partly due to this confinement.

FIGURE 10. Changes in plasma and red cell volumes during pregnancy in two groups of pigs.




9.0
8.0
7.0
6.0
5.0
4.0
3.0
Volume
(l.)
20
40
60
80
100
Days after service.

No decrease in plasma volume in early pregnancy has been reported either in the sheep (Barcroft et al 1939) or in the human, (Hytten and Paintin, 1963), but Taylor et al (1945) reported that in the human, bed rest decreased plasma volume by 15%. If, however, the restriction to the metabolism crates did cause a significant drop in plasma volume, it is surprising that in two of the three animals, 862 and 863, the plasma volumes measured on the 5th and 20th days of pregnancy were identical, (Figure 5). It is unfortunate that no measure of plasma volume was obtained between the 5th and 24th days in No. 861.

When the patterns of change in plasma and red cell volume recorded in Sections I and II are compared they are quite compatible with one another. In Figure 10 the pattern of change in mean plasma and red cell volumes of the two groups of pigs from Sections I and II are compared. It should be noted, however, that the plasma volumes shown for the animals in Section I are reduced by the mean difference between the two groups at 10.5 days, solely for ease of comparison. The results shown for red cell volume are unaltered.

The patterns of change in plasma volume and red cell volume in Section I in which the animals were housed in the dry sow yard with room for exercise, are very similar to those shown by the animals in Section II, in which the animals were confined throughout pregnancy. Although the small numbers of determinations made on each animal in Section I make it impossible to say that the results of the two experi-:ments are the same, it is equally impossible to say that they are different. It should be borne in mind that the animals used in the two experiments were of different parities, and therefore the third parity animals (Section I) may be increasing their lean body masses at differ-:ent rates from the first parity animals.

In general it can be said that although the differences between the results obtained in the present study and those reported in other species/

FIGURE 11. A comparison of the changes in plasma volume during pregnancy in gilts and the increase in the weight of the foetuses and membranes.

o——o mean plasma volume of 3 gilts.

Δ- - -Δ weight of foetuses and membranes from Pomeroy (1960c)

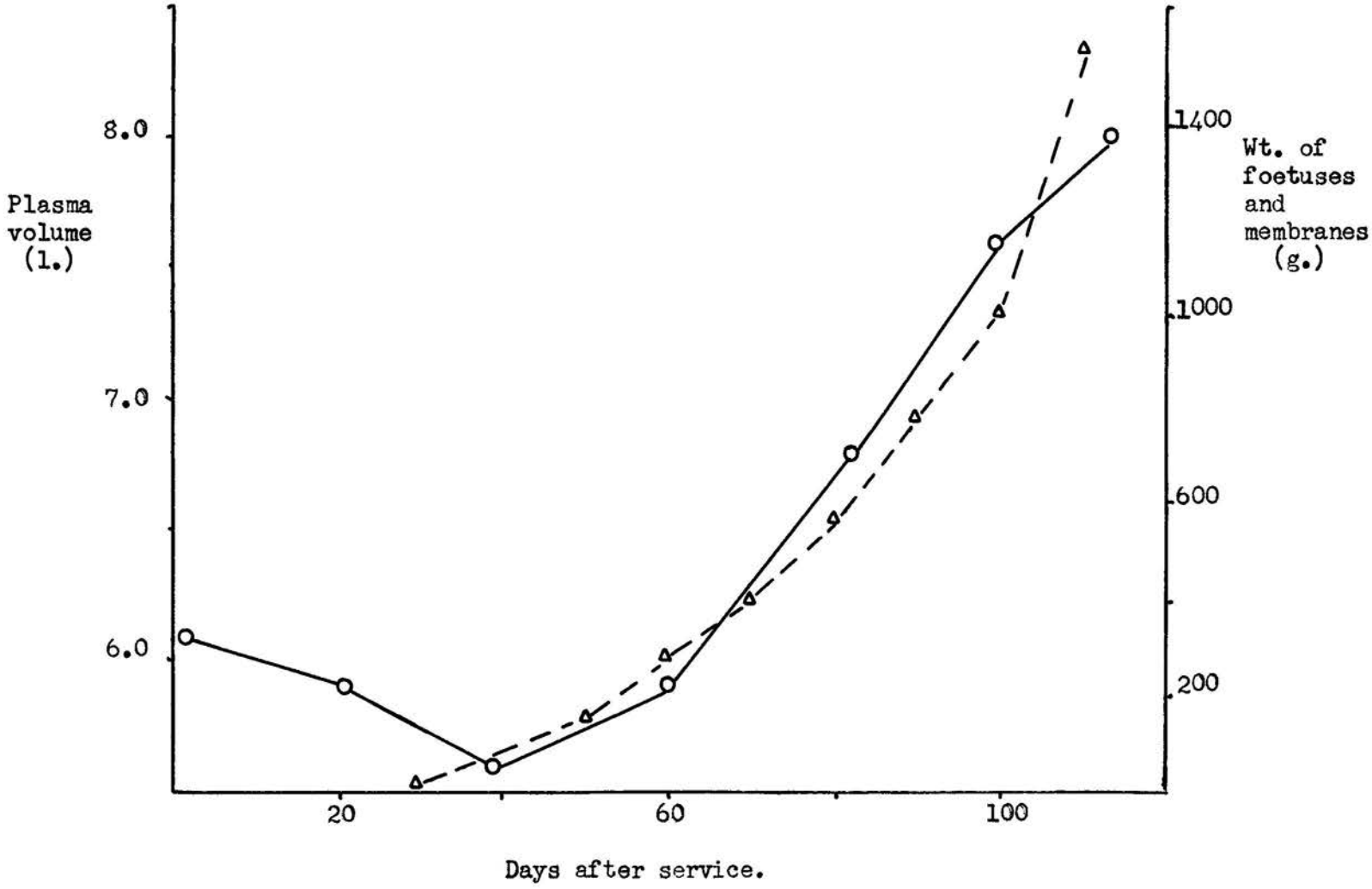
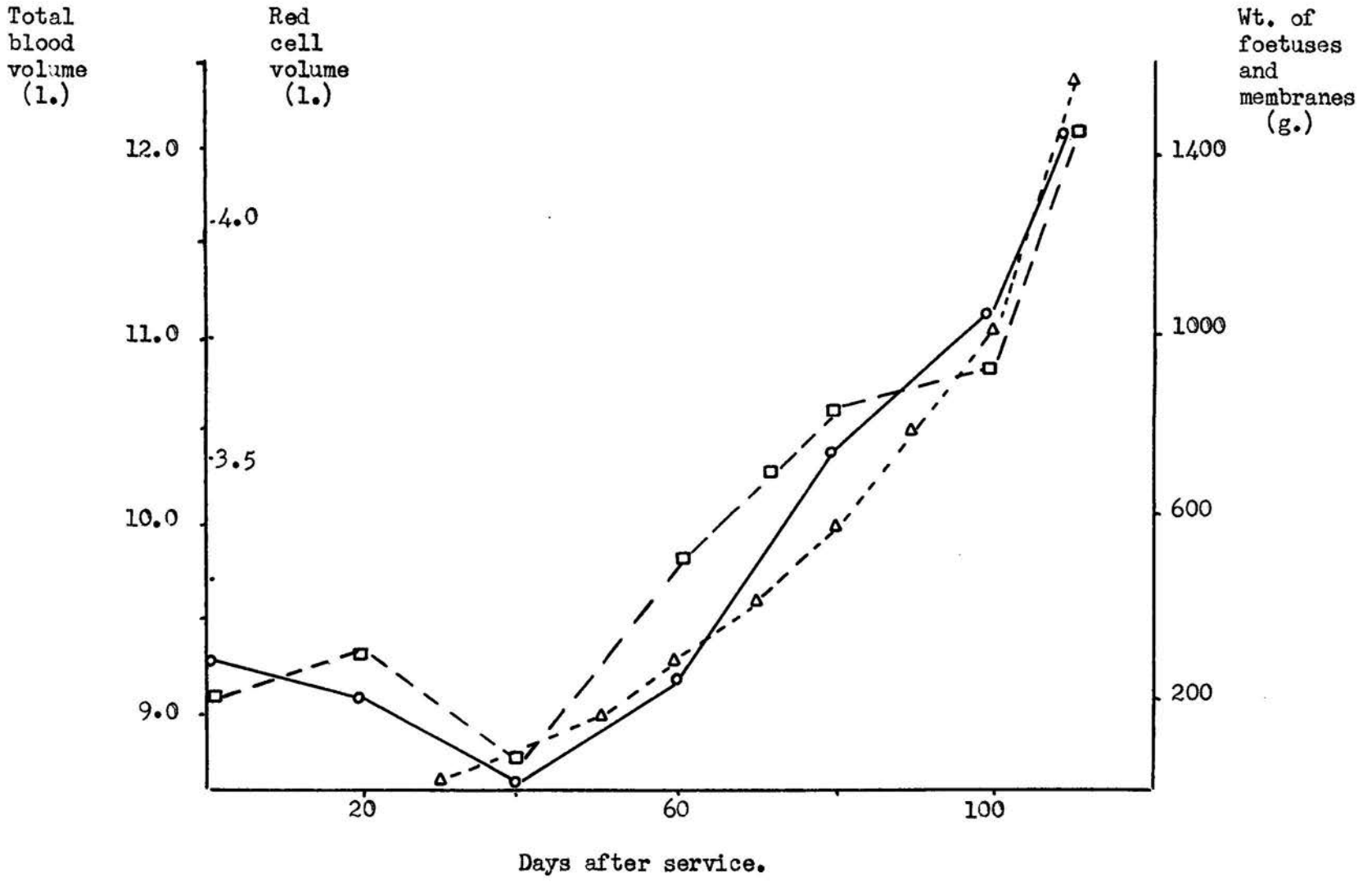

8.0
7.0
6.0
Plasma volume (l.)
1400
1000
600
200
Wt. of foetuses and membranes (g.)
20
60
100
Days after service.

FIGURE 12. A comparison of the changes in total blood volume and red cell volume during pregnancy in gilts and the increase in the weight of the foetuses and membranes.

□– – –□ red cell volume.

o——o total blood volume.

Δ– – –Δ weight of foetuses and membranes from Pomeroy (1960c)


Total blood volume (l.)
Red cell volume (l.)
Wt. of foetuses and membranes (g.)
12.0
11.0
10.0
9.0
4.0
3.5
1400
1000
600
200
20
60
100
Days after service.

species, as well as the known effects of confinement, make it possible that the results were affected by the confinement, it has been impossible to determine whether the results were in fact affected.

b) The pattern of change in plasma volume, red cell volume, haemoglobin and haematocrit during pregnancy. This discussion will be confined almost exclusively to the results of the second experiment, since these are the most detailed. The general trend for both plasma and red cell volumes to increase during pregnancy is in accord with the results obtained in all other species tested. The pattern of this rise appears to differ from that observed in other species, and this is difficult to explain except on the grounds that the animals were confined to crates throughout pregnancy. However, the results obtained in this study indicate that these increases occur in the second half of pregnancy. This would be expected after consideration of the results reported by Pomeroy (1960c) of his studies of the growth of the foetus in utero . In Figures 11 and 12 the mean plasma, red cell and total blood volumes of the three gilts during pregnancy are shown along with the growth curve of the foetuses and membranes reported by Pomeroy. These indicate that regardless of any effect of confinement, both plasma and red cell volumes have increased in step with the growth of the foetuses and membranes. However, since the animals used in this experiment were immature, some of the increases in plasma and red cell volumes were probably associated with increases in maternal tissue.

The lack of any significant decline in haematocrit does not agree with the normal pattern in the human, which shows a decrease (Hytten and Leitch 1964). However, in the case of a human who gave birth to quadruplets (Fullerton, Hytten, Klopper and McKay 1965) the maternal haematocrit increased during pregnancy. Thus the lack of any significant decline in the haematocrit during pregnancy would appear to be a reflection of the larger number of young produced by the sow in each pregnancy/

pregnancy. Although the haemoglobin concentration in the blood shows a quadratic trend, the rise at the beginning of pregnancy and the fall at the end are slight and in general the results of the determinations of haematocrit and haemoglobin concentration are in accord with one another.

These increases, in the plasma and red cell volumes and in the total circulating haemoglobin, will provide increased transport of oxygen and other nutrients to the developing foetuses and it is signif-:icant that the increases in them coincide so closely with the increase in foetal weight. However, it is possible that an increase in plasma volume is not in itself essential to pregnancy but that it is only a re-:flection of a more important increase in the hydration of the maternal tissues. It would be of interest to measure total body water and extra cellular water along with plasma volume.

c) The pattern of change in plasma and red cell volumes, haematocrit and haemoglobin concentration during lactation. The data obtained during lactation deserve some comment, the most obvious being that the number of animals involved in the study was too small for any real conclusions to be drawn. However, there appear to be very strong indications that plasma volume continues to rise after parturition. The maximum plasma volume was achieved after about 14 to 21 days of lactation, which coincides with the period of peak milk secretion by the sow (Lodge 1959). This increase in plasma volume may well be associated with the increased fluid requirements of lactation. It would be of interest to investigate total body water, extra cellular water, and total plasma solids during this period, to throw further light on the question. The decrease in plasma volume after 21 days of lactation may well be associated with the falling off in milk secretion. This increased plasma volume during early lactation is in direct contrast

to/

to the situation which occurs in humans, since in that species the plasma volume drops after parturition, and indeed the volume of plasma 30 days after parturition was considered by Hytten and Paintin (1963) to be the non-pregnant level. However, the amount of milk produced by the nursing human is considerably less than that produced by the lactating sow.

The results of the estimates of red cell volume were less definite in their pattern, but if, as suggested in Section IIc, of this Chapter, they indicate a decline in the red cell volume to a constant level almost immediately after farrowing, this could indicate a decreased oxygen requirement by the lactating sow compared with the pregnant animal. This in turn would support, to some extent, the suggestion that the increase in plasma volume during lactation is associated with the increased fluid requirements of lactation. However, even the lowest estimates of the decreased red cell volume at the end of lactation, 3.5 litres of red cells, are in excess of the expected values in a sow at the beginning of the second parity.

It is obvious that when weaning occurs at 42 days after parturition, there will be considerable adjustments in both plasma and red cell volumes. It is most unfortunate that the experiments could not have been continued to complete the reproductive cycle.

CHAPTER 6. STUDIES TO DETERMINE THE INCREMENTS IN PLASMA VOLUME, RED CELL VOLUME, AND LIVEWEIGHT OF THE SOW DURING PREGNANCY AND TO INVESTIGATE THEIR RELATIONSHIP WITH THE WEIGHT OF THE PIGLETS AT BIRTH.

a) Introduction. The aim of this experiment was first, to determine the mean increases in plasma and red cell volumes during pregnancy in sows and second, to determine whether the birthweight of the piglets was related to the magnitude of these increases. The changes in the sow's liveweight were also studied since it was considered that changes in this parameter might affect the increases in plasma and red cell volumes, independently of any effect of the foetuses.

The data on the increments in plasma and red cell volumes during pregnancy obtained from the experiments described in the previous chapter were insufficient for useful statistical analyses and it was therefore necessary to obtain additional information. This would have to be obtained from measurements made on relatively large numbers of animals which precluded any attempts to measure plasma and red cell volumes at frequent intervals in each animal. It was decided, therefore, to insert a catheter into a vein at the beginning and end of pregnancy in each sow, and to compare the net changes in plasma, and red cell volumes, and liveweight, with both the total weight of the foetuses produced and with the mean weight of the individual foetuses.

b) Materials and methods. Twelve Large White sows in their 4th, 5th and 6th parities were selected. All dietary and experimental procedures were as in the experiments described in Section I of the previous chapter. The piglets were weighed individually within 12 hours of birth.

c) Results. The results, which are shown in Table 16 indicate that plasma volume, red cell volume, and liveweight all increased during pregnancy/

TABLE 16. Gross changes in liveweight, plasma volume and red cell volume in sows between the beginning and the end of pregnancy, and reproductive performance.

Animal No.	Parity	Change in Bodyweight (kg)	Change in Plasma Volume (ml)	Change in Red Cell Volume (ml)	Litter Data Total Weight	Litter Data No.	Litter Data Average Weight
861	1	47.0	1925	1309	15.1	10	1.51
862	1	46.0	1350	996	18.9	15	1.26
863	1	46.0	2207	973	17.5	13	1.34
756	2	37.5	2728	1485	15.5	12	1.30
710	3	30.5	2462	1015	15.9	17	0.93
715	3	24.0	1917	1180	15.1	12	1.26
686	4	12.0	1668	924	14.8	9	1.64
694	4	23.0	1066	365	14.4	12	1.20
632	4	16.0	1365	1129	15.8	14	1.13
726	4	17.0	1807	931	13.3	10	1.33
636	5	17.0	1524	630	11.8	14	0.84
612	6	21.0	1140	214	13.4	10	1.34
Mean		28.1	1763	929	15.1	12.3	1.26
Standard Deviation		± 12.9	± 520	± 368	± 1.9	± 2.4	± 0.22

pregnancy in every animal tested. The mean increase in plasma volume was 1763 ml with a standard deviation of ± 520 ml or approx-:imately 30%. The increment in plasma volume in the older sows (4th, 5th and 6th parities) was less than in the younger sows (1st to 3rd parities). The mean increase in liveweight during pregnancy was 28.1 kg ± 12.9 kg, there being a marked trend for the increment to decrease with increasing parity. The mean increment in red cell volume was 929 ml ± 368 ml. There were however only slight indic-:ations of any trend for this increment to decrease with increasing parity.

Total litter weight was remarkably constant, the mean being 15.1 kg while the standard deviation was ± 1.9 kg or ± 12.6%. The numbers born were less uniform and consequently the mean birthweight was more variable than the total birthweight.

The number of results obtained in this experiment was fewer than had been anticipated, since difficulties in inserting catheters at the end of pregnancy, arose in a number of sows. Unfortunately at this stage there were no further animals available because the Institute herd was being severely reduced in size prior to the establishment of a minimal disease herd.

In the statistical analyses of the results, the total litter weight at birth, (y_1) and the mean piglet weight at birth (y_2) of each litter were examined to ascertain whether they were related to changes in the sow's plasma volume (x_1) red cell volume (x_2) or liveweight (x_3). This was done using simple regression analyses, ignoring the effect of liveweight, and also by taking liveweight simultaneously into account by calculating partial regression coefficients.

No statistically significant relationships were however revealed except that the total litter weight at birth (y_1) was related to the change in/

in liveweight of the sow (x_3)

$$y_1 = 0.10x_3 + 12.3 \qquad \text{R.S.D.} \pm 1.4$$
$$\pm 0.03$$
$$r = +0.69 \qquad P < 0.025.$$

d) Discussion. These results have corroborated the results obtained in Chapter 5 and again demonstrated that plasma and red cell volumes increase during pregnancy in the pig as has also been shown to be the case in all other species so far tested. The tendency for the increment in plasma volume and liveweight to be less in the older sows may indicate that the sows in this particular herd are still increasing their own body tissues until the end of the third pregnancy, and that some of the increment in plasma volume during the first three pregnancies is associated with increasing maternal tissues which are not directly concerned with pregnancy. It has already been suggested for different reasons (Chapter 4) that sows in the Institute herd are not fully grown until the beginning of their fourth parity.

The decrease in the increment in plasma volume during succeed-:ing pregnancies is not in accord with the suggested pattern in women. Hytten and Leitch (1964) suggested that in women, multigravidae almost certainly show greater increases in plasma volume than primigravidae. However, in the countries where the studies on which Hytten and Leitch based this suggestion were made, most women have achieved full adult stature before bearing children, and certainly very few women become pregnant after the second or third ovulation of their lives, as is the case with the pig.

The results of the experiments reported in Chapter 5 indicated that it is probable that the increase in the sow's plasma volume is related to the growth of the foetuses since the maximum increments in both parameters occur at exactly the same time during pregnancy. The failure in this experiment to demonstrate any statistically signif-:icant relationship between the net increments in plasma or red cell volumes/

volumes during pregnancy and either the total weight of the foetuses or the mean weight of the foetuses in each litter may be related to the changes occurring in maternal body composition during pregnancy in the pig.

In order to investigate this possibility further it is necessary to consider the body composition of the sow at the end of pregnancy to decide whether changes in lean body mass could well affect any relationship between the increments in plasma or red cell volumes during pregnancy and the weight of the foetuses produced. The intention of the following argument is to demonstrate this point by calculating the lean body mass (liveweight less fat and the products of conception) and then substituting approximate figures for the volume of plasma associated with each of these tissues.

No studies on body composition were done in the present exper-:iment but such studies were made as part of another series of exper-:iments in which 7 gilts were dissected at the end of pregnancy (Elsley, et al 1966). These gilts received identical treatment to the animals used in the experiments reported in Section II of the previous Chapter, except that they spent only 60 days of pregnancy in metabolism crates. They were slaughtered on the 111th day of pregnancy, the internal organs removed and weighed, the carcass dissected into subcutaneous fat, muscle + intermuscular fat skin and bone. A group of non-pregnant gilts were given similar treatment and slaughtered 111 days after the corresponding heat period to that in which the pregnant gilts were served.

The results of the dissection of the pregnant group were that sub-:cutaneous fat weighed 25 kg, the uterine contents weighed 20.7 kg, and the mean liveweight was 186 kg. By comparison with the results from the dissection of the non-pregnant animals it is possible to calculate that the increases in uterine and mammary tissues due to pregnancy were 5.1 kg and 2.3 kg respectively. The weight of intermuscular fat was esti-:mated to be 7 kg using data from dissection of a similar group of gilts

at/

at the end of pregnancy, (Elsley, Anderson and McPherson, unpublished data).

When the weights of fat and the products of conception (foetuses, placentae, fluids, and increased uterine and mammary tissue) are sub-:tracted from the liveweight at the end of pregnancy then the weight remaining in this case 126 kg is equivalent to a non-pregnant animal with no fat.

The model animal is now made up almost entirely of lean tissue and were the plasma and red cell volumes of this animal known a figure for plasma and red cell volumes per kilogram of'lean body mass' could be derived.

Since the animals used in the experiments described in Section II of the previous chapter were so similar to those used in the slaughter experiment it can be assumed that they also had a 'lean body mass' of 126 kg and that the increases in uterine and mammary tissues were also 5.1 kg and 2.3 kg respectively.

The estimated body composition of the gilts used in the studies reported in Chapter 5 Section II is therefore as follows

Lean body mass	126.0 kg
Weight of foetuses	17.2 kg (Table 16)
Weight of placenta	3.0 kg
Increase in uterus	5.1 kg
Increase in mammary tissue	2.3 kg
Total	153 kg

However the liveweight of these animals was 193 kg therefore the remainder of the body weight at the end of pregnancy must be fatty tissue and foetal fluids

$$\therefore \text{ Weight of fat} = 193 - 153 - 2 \text{ kg fluids}$$
$$= 28 \text{ kg}$$

From the results of the experiments reported in Chapter 4 (Blood volume in the first two weeks of pregnancy) it is possible to make/

make an estimate of the volume of plasma and red cells associated with each kilogram of the tissues mentioned above. It has been stated that if the plasma and red cell volumes of the model pig con-:structed from the results of the slaughter experiment were known, then it would be possible to estimate the volume of plasma assoc-:iated with each kilogram of 'lean body mass'. In the results of the experiments reported in Chapter 4 there were some animals which weighed only 140 kg at the beginning of their 4th, 5th or 6th pregnancies. These animals were very thin indeed and it is un-:likely that their bodies contained much fatty tissue and they were therefore very similar to the model animal. Using the equations relating plasma volume and liveweight in the 4th, 5th and 6th parities the plasma volume of a sow weighing 140 kg should be 7100 ml which is approximately 50 ml per kilogram of (lean) body mass. A figure of 23 ml red cells/kg of lean body mass can be derived in a similar manner.

The derivation of a figure for fatty tissue is more difficult. However, the difference in the plasma volume between a sow weighing 140 kg and one weighing 220 kg will be 1040 ml (Table 9). Therefore 80 kg of tissue are associated with 1040 ml plasma. It is unlikely that this tissue consists entirely of lean tissue as this would require an additional 4000 ml of plasma. If the figure of 50 ml plasma/kg of 'lean body mass' is valid, and 19 kg of the additional tissue were lean, then each kilogram of fatty tissue would be associated with 1 ml of plasma. If the additional tissue were all fat then each kilogram of fatty tissue would be associated with 1040/80 = 13 ml plasma. It is unlikely that all the additional tissue is fat (1.5 kg must be blood) but no figures for the carcass composition in this weight range are available. The figure for the plasma associated with 1 kg of fat must lie within this range (1 - 13 ml/kg). The actual figure within this range will make only slight differences to the final calculations, the/

the figure of 10 ml plasma/kg of fat has been adopted because it was considered that the actual figure would be on the high side of the mean. The figure of 5 ml red cells /kg of fat can be derived in a similar manner.

There are no figures available for the volume of plasma and red cells associated with each kilogram of the products of conception but since all these tissues are lean tissue, (except the foetal fluids), then the figures derived for lean body mass are the only suitable figures available.

An estimate of the plasma and red cell volumes associated with the various tissues of the bodies of the gilts used in the previous experiment (Chapter 5) can now be made. This is shown in Table 17.

TABLE 17. The volume of plasma and red cells associated with some tissues at the end of pregnancy in gilts.

Tissue	Weight (kg)	Plasma /kg (ml)	Red Cell /kg (ml)	Plasma Volume (ml)	Red Cell Volume (ml)
lean body mass	126	50	23	6300	2898
foetuses	17.2	50	23	860	396
placentae	3.0	50	23	150	69
increased uterine tissue	5.1	50	23	255	117
increased mammary tissue	2.3	50	23	115	53
fatty tissue	28.0	10	5.0	280	140
			Total ..	7960	3653

In the Table no allowance has been made for plasma or red cells associated with foetal fluids, since these are unlikely to require any.

The estimated plasma volume of 7996 ml is very close to the measured plasma volume of 8000 and even if the true figure for the plasma associated with fat lay at either of the two possible extremes, the estimate would vary from only 7716 ml to 8080 ml. Since the

largest single component of the total plasma volume was the plasma associated with the 'lean body mass' then the estimate of 50 ml of plasma/kg lean was not unreasonable.

However the estimate of 126 kg lean tissue was the mean of 7 animals and since it is unlikely that all these animals began pregnancy with the same 'lean body mass' or increased their 'lean body mass' at the same rate throughout pregnancy, the standard deviation around this mean could easily be $\pm$ 5% or $\pm$ 6.3 kg lean. If 'lean body mass' and the products of conception are associated with the same or even similar amounts of plasma then since the standard deviation around the mean of the total birthweight (the largest component of the products of conception) was only $\pm$ 1.9 kg, the variation about the mean increase in 'lean body mass' during pregnancy, will have a far greater effect on the variation around the mean of the increases in plasma volume than the variations in total birthweight.

The possible effects of changes in maternal body composition were realised at the beginning of this experiment but it was expected that the inclusion of a term based on liveweight changes in equations relating plasma volume with birthweight would reduce these effects, especially in mature sows, where the changes in maternal body composition would be minimal.

When the data for the mature sows used in this experiment are treated in a similar manner to the data on the gilts, some other relevant facts emerge.

The mean total weight of the foetuses produced in each litter was 13.9 kg. No figures are available for the weights of placentae or fluids or for the increase in the weights of uterine and mammary tissues but there are unlikely to be major differences between the gilts and the mature sows. Therefore the total weight gain due to the/

the products of conception can be calculated.

Weight of foetuses	13.9 kg
Weight of placentae	3.0 kg
Increase in uterus	5.1 kg
Increase in mammary tissue	2.3 kg
Total	24.3 + 2 kg fluids.

The mean increase in liveweight of this group was only 17.7 kg and there is therefore a discrepancy of 8.6 kg of tissue. This could be a simple loss of fat, but Salmon-Legagneur and Jaquot (1961) showed that the mature sow could show a greater increase in her lean tissue during pregnancy than the non-pregnant sow fed the same ration. Lodge, Elsley and McPherson (1966) suggested that in some conditions fat may decrease and be replaced by tissue of a lower energy content during pregnancy.

In the present experiment the products of conception (less fluids) weighed 24.3 kg which would be associated with 24.3 x 50 = 1215 ml increment in plasma. A loss of fat of 11.6 kg and a gain in lean of 3.0 kg would represent a total increment in plasma volume of (3.0x50) - (11.6 x 10) = 34 ml.

The total increment in plasma would then have been 1215 + 34 = 1249. These figures for gain in lean and loss of fat have been selected to give an answer close to the measured value, but it is realised that a net loss of 8.6 kg of tissue could represent a number of different possible combinations of loss fat and gain in lean. If then the same change in liveweight can represent a number of different changes in the 'lean body mass' then liveweight change in pregnancy is not a very useful predictor.

The criticisms of the arguments presented in this chapter will now be considered. There is for example no evidence to support the suggestion that a figure of 50 ml plasma/kg applies to the foetuses. The foetuses might need considerably more or they might need less

than this since they have their own blood supply. This situation is unlikely since their only source of nutrients is the maternal blood supply, and it has been shown that the nutrient and oxygen requirements of a tissue influence the plasma and red cells associated with it. (Kjelberg Rudhe and Sjö strand, 1949).

It is also possible that the figure of 50 ml plasma/kg of lean body mass applies only to animals similar to the ones from which it was derived. If for example a tissue, such as the alimentary canal, required a very large volume of plasma and red cells and the weight of the alimentary canal did not alter greatly between light and heavy sows, then the amount of plasma associated with each kilogram of 'lean body mass' would be less in heavy sows.

The results of the experiment reported in this chapter have failed to demonstrate any significant relationship between the increment in plasma and red cell volume during pregnancy and the weight of the foetuses produced in that pregnancy. However the results reported in Chapter 5 indicated that the plasma and red cell volumes increased at the same time as the weight of the foetuses increased suggesting some relationship. The failure in the present experiment to demonstrate any correlation seems likely to be related to an inability to differentiate between increases in plasma volume related to changes in the maternal body composition and those related to the growth of the products of conception. It is possible that by measuring the increment over another period such as 40 - 110 days of pregnancy a correlation might be demonstrated, especially if the changes in maternal body composition occur during the early stages of pregnancy, as has been suggested by Salmon-Legagneur and Rerat, (1962). An alternative is to find a more suitable indicator of changes in 'lean body mass' than the changes in liveweight, to allow their effect on plasma and red cell volume to be taken into account more accurately.

CHAPTER 7. THE RELATIONSHIP BETWEEN HIGH BIRTH WEIGHT AND MATERNAL DIABETES.

a) Introduction. The role during pregnancy of the hormones associated with carbohydrate metabolism is complicated and as yet unresolved. However, it is known that Diabetes mellitus, a condition associated with an imbalance of these hormones, is associated in human pregnancy with high foetal birth weights.

In a review, "Diabetes and Pregnancy", Kyle (1963) states that in general the infants of diabetic mothers are larger at birth than off-:spring of the same gestational age of normal mothers. Miller (1956) reported that 25% of the infants of a series of diabetic and prediabetic mothers exceeded 4,000 g weight at birth, a weight achieved by only 0.5% in the general infant population, while Cook, O'Brien, Hansen, Beem and Smith (1960) compared a series of 19 infants from diabetic mothers with the normal growth curves and concluded that the infants of diabetics were heavier and larger than normal. This difference may only become apparent in late pregnancy, since Gellis and Hsia (1959) concluded that until 28 weeks gestation the weights of infants of diabetic and non-diabetic mothers were similar, but thereafter the infants of diabetics grew larger and were on average 250-500 g heavier at birth. Pedersen (1959) reported that on average the infant of the diabetic is 1.5 cm longer than the gestational age would indicate. Gollen, Ellis and Evans (1958) performed glucose tolerance tests on 29 women who had produced babies over 4,000 g in weight. Of these women, 14 had some evidence of the diabetic state, either abnormal glucose tolerance curves (11) or elevated blood glucose (3).

Not only is the production of large babies an indication of diabetes which has already manifested itself, but it is an indication of the possible presence of prediabetes. This condition is difficult to define but can be described as the period when there are no clinical signs of diabetes in those/

those people who will in the future develop diabetes. Thus a history of heavy babies is likely to be an indication that at some time in the future diabetes will develop. Jackson (1952) showed that 62% of women who developed diabetes after childbearing age had produced at least one child over 4,500 g. In another study (Kriss and Futcher, 1948), 77% of women who produced infants over 4,500 g in weight developed clinical diabetes at a later date. Conn and Fajans (1961) reported that no fewer than 96% of mothers who produced infants larger than 5,800 g later developed diabetes. There is also a tendency for the weight of babies to increase as diabetes approaches (Paterson and Burnstein, 1949; Hagbard, 1958; Paton, 1948).

From the preceding evidence it is apparent that the incidence in women of very heavy babies can often be related to the presence of clinical diabetes or the appearance of the condition at a later date. However, it must be pointed out that not all diabetic women produce heavy babies and some produce very small babies. Diabetes which has been present over a number of years tends to produce a number of conditions, principally vascular disease. Kyle (1963) states that the majority of young women with long-standing diabetes develop premature atherosclerosis in addition to diabetic angiopathy involving retinal and renal vessels. In the pregnant diabetic the foetal size is related to the presence or absence of vascular disease (Given and Tolstoi, 1957; Sexton, 1959; Given, 1959; Cardell, 1953; Gellis and Hsia, 1959; Oppe, Hsia and Gellis, 1957; Hsia and Gellis, 1957), the foetus being smaller in the presence of vascular disease.

Although there are no reliable reports of naturally-occurring diabetes in the adult pig, it was decided to investigate the possibility that decreased glucose tolerance was related to birth weight in the pig.

b) <u>The glucose tolerance test.</u> The diagnosis of Diabetes mellitus in the human is largely dependent on the result of a glucose tolerance test. /

test. This consists of evaluating the rise and fall of blood glucose concentration after the administration of glucose to the animal. The route by which this glucose is infused has given rise to two distinct methods of testing glucose tolerance, the oral and the intravenous. Intravenous glucose tolerance tests can be further divided into the rapid infusion test, where a load is infused over a short interval (2 - 4 mins), and the constant infusion test in which the glucose is infused slowly over a period of 1 - 2 hours.

The result of an oral glucose tolerance test is difficult to interpret on a quantitative basis as it consists of two main compon-:ents, the inflow of glucose from the alimentary canal and the removal of glucose by the tissues. This results in a period when the blood glucose concentration is rising followed by a period when it is falling. In spite of this the test is sufficiently precise to pick out gross abnor-:malities. Recently, however, it has been postulated (McIntyre, Holdsworth and Turner, 1964) that a humoral substance is liberated from the jejunal wall during glucose absorption and that this acts, along with a rise in blood glucose, in stimulating the release of insulin from the islet cells. On the basis of this observation it is possible that the oral glucose tolerance test gives a more useful picture of normal carbohydrate metabolism.

Of the two methods of determining intravenous glucose tolerance, the rapid infusion method is the more widely used, probably because it is the more easily interpreted, since it contains only one function, the removal of glucose from the bloodstream. On the other hand, it is claimed that using the constant infusion intravenous glucose tolerance test, the "unphysiologic" high blood glucose concentrations associated with the rapid infusion test are avoided (Hlad, Elrick and Witten, 1956). However, whether any one of these methods is more "physiologic"/

"physiologic" than the other is a matter of personal appraisal, since, as the next section will show, not enough is known about which para-:meters are in fact being measured by the glucose tolerance test.

It was decided to use the rapid-infusion intravenous glucose tolerance in the pig for two reasons;

(a) Because this test provides a simple quantitative measure-:ment.

(b) Because the pig is thought to have a delayed gastric emptying time (Dukes, 1942). This would make the interpretation of an oral glucose tolerance test very difficult.

CHAPTER 8. THE PHYSIOLOGY OF THE INTRAVENOUS GLUCOSE TOLERANCE TEST.

a) The diffusion of injected glucose into the extracellular water. The intravenous glucose tolerance test is a method whereby the rate of loss of a load of glucose from the blood can be assessed without the complication of continuing entry of glucose to the blood from the alimentary canal. However, the rate of loss of glucose from the blood is not a simple process. Initial losses of glucose from the blood take place by simple diffusion of glucose into a portion of the body fluids roughly equivalent to the extracellular water (Frankson, Ooms, Bellens, Conrad and Bastenie, 1962). During the period of high blood glucose concentrations the renal threshold may be exceeded and some glucose lost to the urine. In about 10 minutes (Frankson et al, 1962) the glucose load is distributed throughout the extracellular water and there is no further loss of glucose from the blood by this route.

b) The uptake of glucose by muscle tissue. From this point the loss of glucose from the bloodstream is due almost entirely to uptake of glucose by the cells of the body. The rate of uptake of glucose by peripheral tissue is directly proportional to the arterial glucose concentration up to concentrations of 500-600 mg/100 ml (Lang, Goldstein and Levine, 1954). This was demonstrated using the perfused hind limbs of an intact anaesthetised dog. When the experiment was repeated using previously depancreatised dogs, almost the same maximum rate of glucose uptake was achieved by the tissues of the hind limb, but at a much higher arterial glucose concentration (1000 mg/100 ml). Unfortunately no details of the rates of uptake at lower arterial glucose concentrations (100 - 300mg / 100 ml) in the pancreatectomised dogs were given. There was therefore no information on whether, at blood glucose concentrations achieved in the intravenous glucose tolerance test, the relationship between arterial glucose concentration and the rate of tissue uptake was direct, /

direct, or whether a secretion of insulin in response to high blood glucose concentration was involved.

Little is known about the effect of a sudden increase in blood glucose concentration in vivo on the secretion of insulin from the pancreas. Coore and Randle (1964), using incubated rabbit pancreas, showed that insulin secretion was accelerated by increasing medium glucose concentration above 35-70 mg/100 ml. Grodsky, Batts, Bennett, Viella, McWilliams and Smith (1963), using isolated rat pancreas, showed that with a medium concentration below 50 mg/100 ml, glucose insulin secretion was negligible, but above that figure insulin secretion increased with increasing concentration, up to a glucose concentration on the medium of 500 mg/100 ml.

Seltzer (1962), using intact dogs, showed that a rapid infusion of 15 g of glucose into the femoral vein over five minutes increased the plasma 'insulin-like' activity'(ILA) of the pancreatic effluent plasma by a factor of almost seven. On the other hand, Kosaka, Ide, Kuzuya, Miki, Kuzuya and Okinaka (1964), also using intact dogs, found no increase in plasma 'insulin-like' activity' of pancreatic effluent plasma after glucose loading, until the blood glucose had returned to normal concentration. Antoniades, Gundersen and Pyle (1961) found, in non-diabetic humans, that after intravenous injection of glucose the free (active) insulin increased markedly, while the bound (inactive) insulin decreased. After an intravenous injection of glucose in normal humans, Frankson et al (1962) found an increase in plasma 'insulin-like activity' in the region of 50%. On the basis of the observation that the rate of loss from the blood of previously injected C^{14} glucose was the same before and after glucose administration, Frankson postulated that this increase in plasma ILA, although statistically significant, had not significantly increased the rate of uptake by the tissues, and that the rate of removal of a sudden intravenous glucose load was dependent on the level of circulating plasma ILA at the time of glucose injection.

However, /

However, it is unwise to compare rates of removal of glucose on a percentage basis when the size of the body pool alters from normal after an intravenous load of glucose. Thus, if after an intravenous glucose load, the body pool is 40 g of glucose and the rate of removal K = 1% per minute, then the tissues are removing 0.4 g/min. If in the steady state the body pool is 15 g and K = 1%, then the tissues are consuming 0.15 g per min.

It seems likely that an intravenous load of glucose stimulates insulin secretion which will in turn affect the rate of membrane transport of glucose. The regulation of glucose uptake by muscle is a complex process and involves numerous factors in addition to the level of insulin and the concentration of glucose in the surrounding medium. These additional factors include 1) the rate at which glucose is phosphorylated to glucose-6-phosphate within the cell, 2) other hormones, such as growth hormone, corticosteroids, adrenaline, thyroxine and glucagon, 3) the availability of oxygen and 4) the presence of other substrates such as ketone bodies and fatty acids (Randle and Morgan, 1962). Although plasma ILA and arterial glucose concen-:tration are the factors apparently most affected by a sudden intra-:venous load of glucose, little is known about the effects on the other factors by a sudden intravenous load. It is likely that the condition of the tissues with respect to all these factors immediately prior to the infusion of the glucose load will affect the response of the tissues to the glucose.

c) <u>The effect of an intravenous load of glucose on the liver</u>. The rate at which a sudden intravenous load of glucose is apparently removed from the blood will be governed to some extent by the activities of the liver. The most important point to be considered is whether or not the liver continues to release glucose to the circulation after a sudden/

sudden intravenous load of glucose. Measurements of hepatic glucose output can only be made directly when the flow of blood through the liver and the glucose concentration gradient over the liver are known. Blood is supplied to the liver by both the portal vein and the hepatic artery and is, therefore, not homogeneous; in the liver of the un-:anaesthetised dog 80% of the incoming blood enters through the portal vein, the remaining 20% through the hepatic artery (Blalock and Mason, 1936). Thus by using hepatic vein catheters and arterial catheters to measure hepatic outflow Frankson et al (1962) were in fact measuring the net splanchnic output.

The advent of C^{14} glucose has made it possible to measure the rate of hepatic glucose output without using catheters. A small quantity of C^{14} - labelled glucose is injected into the bloodstream and the rate of decline of specific activity of blood glucose is measured. As the decline in specific activity is a measure of dilution and the only source of endogenous glucose is the liver, then the rate of decline of specific activity is a measure of hepatic glucose output (Searle and Chaikoff, 1952). When a dose of glucose is suddenly introduced intravenously the specific activity of blood glucose drops by dilution and then remains constant. This plateau is regarded by some authors as indicating that hepatic glucose outflow has ceased and the labelled glucose is not being diluted (Searle and Chaikoff, 1952). Reichard, Freidman, Maass and Weinhouse (1958), in the dog and Frankson et al (1962) in the human used this technique and both groups concluded that an intravenous infusion of glucose inhibited hepatic glucose output during hyperglycaemia in these species.

Using an eviscerated dog, and a constant infusion pump to replace the liver, Steele (1959) showed however that a plateau in specific activity was established after rapid intravenous infusion of glucose/

glucose even when the infusion pump was feeding glucose into the blood at a constant rate. Steele and Marks (1958) used a priming dose of C^{14} glucose followed by a constant infusion of C^{14} glucose designed to balance exactly dilution of glucose C^{14} by endogenous glucose in the fasting animal. An intravenous load of glucose was then administered with sufficient C^{14} glucose present to give the same specific activity to the load as was present in the blood. Continuous infusion of C^{14} was continued and hence cessation of hepatic glucose output would have resulted in a rise in the specific activity of blood glucose. No such rise occurred, and it was concluded that hepatic glucose output had continued. A similar finding, using the same technique, was reported by De Bodo, Steele, Altszuler, Dunn, Armstrong and Bishop (1959).

Steele, Bishop, Dunn, Altszuler, Rathgeb and De Bodo (1965), using the constant C^{14} infusion technique, showed that exogenous insulin and glucose, both reduced endogenous glucose production by 50% during the first hour after infusion of glucose and insulin began.

d) <u>The interpretation of intravenous glucose tolerance tests.</u> In general it can be said that the response of an animal to a sudden intravenous load of glucose is governed by two main factors, the rate of removal of excess glucose by the tissues and the glucose balance of the liver. Although the uptake of glucose by the tissues is affected by a number of factors, the most important is probably the level of circulating active insulin. No convincing evidence either for or against the secretion of insulin by the B cells of the pancreas in response to a glucose load has been presented. The response of the liver to a load of glucose is also of importance, but again no convincing evidence has been presented either for or against the cessation of hepatic glucose output during hyperglycaemia.

The confused state of knowledge on the physiological response to a sudden intravenous load of glucose is reflected in the controversy on the/

the most suitable method of interpreting the results of an intravenous glucose tolerance test. These differences in interpretation of the test can best be described by listing the workers and their suggested methods of interpretation.

Jorgensen (1926) and Ross (1938) used the area under the curve of observed glucose values against time as a measure of glucose tolerance. Lozner, Winkler, Taylor and Peters (1941) gave a standard dose of 25 g glucose and used the blood glucose concentration two hours after injection as the measure of glucose tolerance. Allibone and Tunbridge (1939) and Tunbridge and Allibone (1940) infused 27.6 g of glucose in three minutes and used the time taken for the blood sugar concentration to return to the fasting level as the measure of glucose tolerance.

In recent years the glucose tolerance test has been interpreted on the basis of the observation by Fishberg (1930) that the rate of removal of intravenously injected foreign sugars such as xylose and pentose was exponential with regard to time.

Hamilton and Stein (1942) gave an intravenous load of 0.5 g glucose /kg body weight to humans and found that the rate of removal of glucose was approximately exponential between 200 and 100 mg %. The slope of the regression of the logarithmof blood glucose concentration on time was used as the index of glucose tolerance.

Greville (1943) found that the rate of removal of excess glucose was a first order reaction, tending not to zero but to a positive concentration of blood glucose. This positive concentration of glucose varied from test to test.

Amutzio, Stutzman, Vanderbilt and Nesbitt (1953) performed intravenous glucose tolerance tests on humans and concluded that the removal of glucose tended not to zero but to the fasting level.

Duncan (1956) interpreted a series of intravenous glucose tolerance tests in two ways, either assuming that the removal of glucose tended to zero/

zero (Total index) or towards the fasting level (Increment index). He found both tests repeatable but that the Increment index did not grade a series of subjects in the same order of glucose tolerance as the Total index. Furthermore, he found that the Increment index was repeatable regardless of the initial dose, while the Total index was affected by dose. However, when the Total index was used in two separate trials on the same group of people, the only difference being that the glucose load was 25 g in one trial and 50 g in the other, the 25 g trial graded the subjects in the same order as the 50 g trial.

Hlad, Elrick and Witten (1956) proved mathematically that after intravenous glucose the blood glucose values must tend towards a steady value. They then used the figures derived from a series of tests and extrapolated to infinite time. The blood glucose, however, did not tend to the fasting glucose concentration or to zero but to a value between them.

Frankson et al (1962) attempted to prove that the fall of blood glucose after an intravenous glucose load was made up of three simple and separate phases, distribution of the load throughout the extracellular water, removal of excess by the tissues and the liver, and the resumption of hepatic glucose output at or above the fasting level. During the second phase they considered that the rate of glucose removal was related to absolute, and not increment, blood glucose values. Unfortunately the proof of this thesis depended on two methods of measuring hepatic glucose output, which are open to criticism, the hepatic vein catheter technique and the single injection C^{14} technique, and also on the assumption previously mentioned that a significant increase in circulating plasma 'insulin-like activity' had no significant effect on the rate of uptake of glucose by the peripheral tissues.

It is impossible to say without further clarification of the physiology of the intravenous glucose tolerance test which method of interpretation is most suitable. Duncan (1956) considered that since the

Increment /

Increment index was repeatable regardless of dose, it was the most suitable interpretation. Unfortunately this approach is purely empirical since, as the physiology of the intravenous glucose tolerance test is not fully understood it is not possible to say whether the response to varying dosage levels should or should not be identical. At the same time it should be pointed out that it is unlikely in the case of all the previously mentioned papers that the removal of glucose does tend to zero since the method of analysis for glucose included a certain amount of reducing substances other than glucose. The concentration of these substances in blood does not alter during the test (Amutzio, 1953).

In conclusion it must be stated that the intravenous glucose tolerance test is an imprecise measure of carbohydrate metabolism. It will pick out pathological states, but, in the normal organism, so many factors affect the test, that any measurement of glucose tolerance could be the result of any one of a large number of possible situations. Indeed in the normal animal the only interpretation which can be put on the results of a test is that the animal is either more or less able to remove an intravenous load of glucose.

CHAPTER 9. INTRAVENOUS GLUCOSE TOLERANCE IN PIGS.

Section I. The methods used.

a) The measurement of intravenous glucose tolerance in sows. When formulating a routine intravenous glucose tolerance test for use in sows, the initial problem was whether to inject a standard amount of glucose, regardless of the liveweight of the animal, or whether to use a dose based on the animal's liveweight. Little evidence was available to support either method. Hamilton and Stein, (1942) and Frankson et al (1962) gave an injection of glucose based on liveweight, while Tunbridge and Allibone (1941) Amutzio et al (1953), Hlad et al (1956) and Duncan (1956), all gave a standard load of glucose, regardless of liveweight.

It was decided to use a standard dose of 50 g per animal regard-:less of liveweight on the basis of the following considerations.
(1) Liveweight is a poor basis, as any one weight may represent vary-:ing combinations of tissues, especially muscle and fat, which meta-:bolise glucose at different rates under the same set of conditions.
(2) It was hoped that an injection of 50 g of glucose would raise blood glucose concentration to a point above the renal threshold in the pig, 142-158 mg/100 ml blood (Link, 1953b). Under these conditions it was anticipated that a large portion of the excess above the renal threshold would thus be removed from the blood through the kidneys. The renal threshold would then tend to set a limit to the quantity of glucose presented to the tissues for removal.

The test which evolved was thus identical with that described by Duncan (1956) for use in humans. The pig was fasted overnight and three samples of blood were removed for estimation of fasting blood glucose concentration. An injection of 100 ml of 50% glucose solution was then made over a period of 4 minutes. Blood samples were removed at 5 minute intervals until 30 minutes after injection, and at 10 minute intervals thereafter until the sampling was stopped.

All/

All injections were made and all blood samples were taken through the indwelling polyethylene catheters described in Chapter 3. All blood samples were therefore samples of venous blood.

b) The measurement of blood glucose. There are available a number of methods of measuring blood glucose. Almost all depend on the reduction by the blood glucose either of an alkaline copper sulphate solution or of an alkaline potassium ferricyanide solution. The amount of the reduction and hence the amount of reducing substances present is determined either iodometrically or colorimetrically. These methods measure reducing substances rather than glucose specifically, and there may be present in blood other reducing substances in amounts equivalent to 20-30 mg glucose/100 ml blood. (Varley, 1960). In order to measure true blood glucose it was decided to use the glucose oxidase-peroxidase method described by Huggett and Nixon (1957). In the presence of glucose oxidase, which is highly specific for glucose, glucose is oxidised to gluconic acid and hydrogen peroxide. The hydrogen peroxide can, in the presence of peroxidase, oxidise a suitable oxygen acceptor (o-dianisidine) to give chromogenic oxidation products. The intensity of colour developed is proportional to the amount of glucose initially present. The method of Huggett and Nixon (1957) was modified in that using BDH glucose oxidase and peroxidase, 50 mg glucose oxidase/100 ml oxygen acceptor reagent was found to be sufficient, as opposed to 125 mg. The method was further modified by using 'tris' ♯ buffer instead of sodium dihydrogen phosphate - sodium hydroxide buffer.

The anti-coagulant used was a mixture containing 10 mg sodium fluoride and 30 mg potassium oxalate per 10 ml blood, to prevent glycolysis (Bayliss, 1950). This was added to the sample bottles as 1 ml of solution and the bottles were then dried in the oven at 100° C.

The blood was deproteinised using 10% zinc sulphate and 0.5 N sodium/

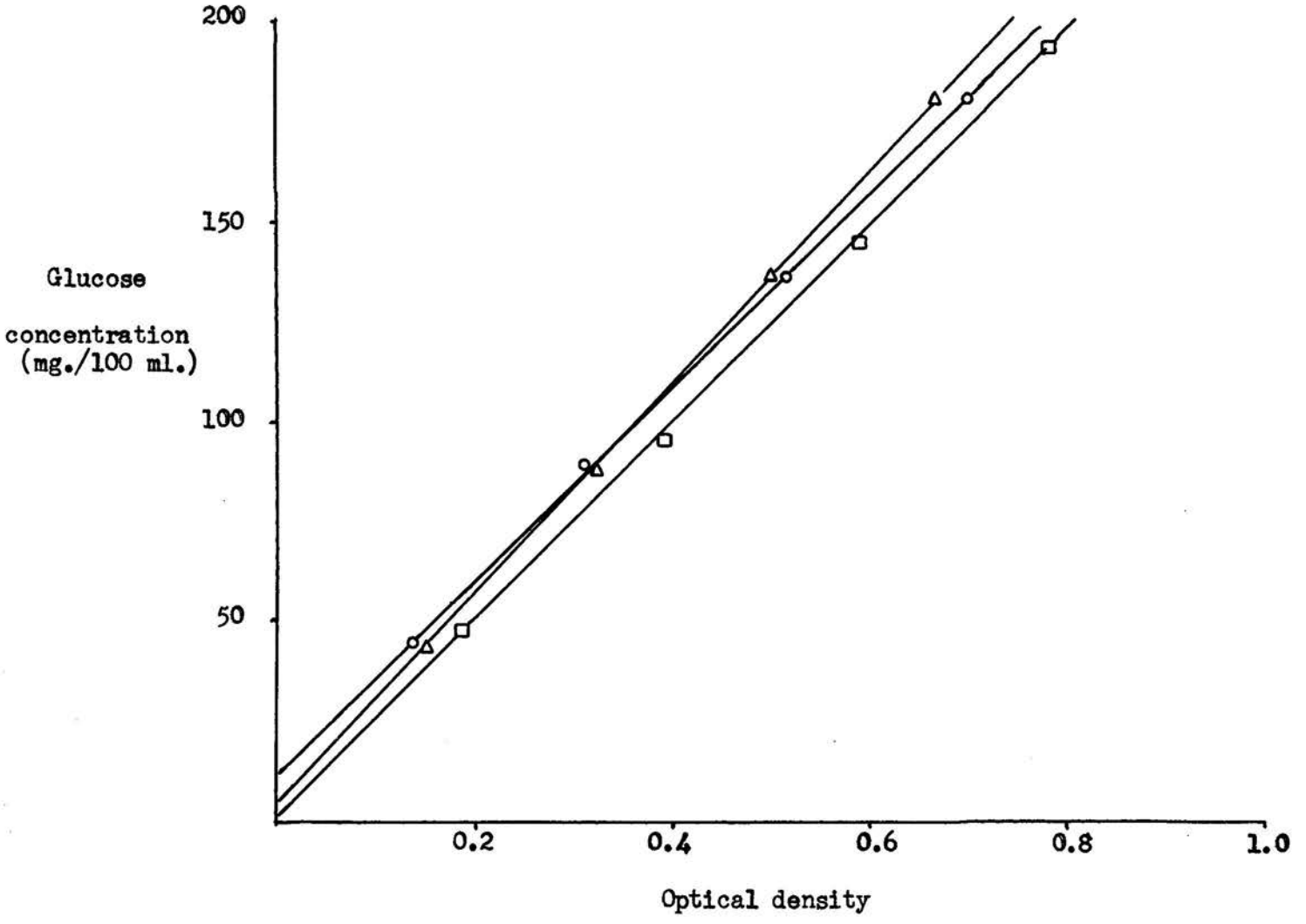
♯ 2 - Amino - 2 - (Hydroxy-Methyl) - Propane - 1 : 3 Diol, B. D. H. Ltd.

FIGURE 13. The relationship between glucose concentration in the solution and optical density of the incubated reagent.

o on day 1.

Δ on day 2.

□ on day 3.


200
150
100
50
Glucose
concentration
(mg./100 ml.)
0.2
0.4
0.6
0.8
1.0
Optical density

sodium hydroxide.

The method was tested to determine whether Beer's Law was obeyed over the range of concentrations of blood glucose expected (50 - 200 mg./ 100 ml blood). It was found that the relationship between the colour produced in the reaction and the concentration of glucose in the sample was linear over this range. However the relationship was of the form

$$y = bz + c$$

where y = glucose concentration

z = optical density

and c = a positive value of y.

Unfortunately neither c nor b (the regression coefficient) were constant (See Figure 13). To overcome this, two standards (50 mg/100 ml and 150 mg/ 100 ml) were carried for every determination and the glucose concentration of the unknown calculated from the following equation:-

$$\text{Blood glucose} = z + \frac{D_u - D_z}{D_y - D_z} \times (y - z)$$

where z = glucose concentration of 50 mg % standard in mg/100 ml

y = glucose concentration of 150 mg % standard in mg/100 ml.

D_u = optical density of the unknown

D_z = optical density of the low standard

D_y = optical density of the high standard

The concentration of the two standards was chosen so that the bulk of the expected blood glucose values would fall between them. To reduce any further chance of error all blood samples and standards for any one test were analysed together using the same batch of oxygen acceptor reagent and incubated for exactly the same time.

Standards were made up as follows. Approximately 1 g of glucose was dissolved in distilled water and made up to 100 ml. Five ml and 15 ml of this solution were then pipetted into volumetric flasks which were then made up to 100 ml with distilled water. The concentration

of/

of glucose in these flasks was then 50 mg/100 ml and 150 mg/100 ml respectively. The contents of these flasks were then treated as blood samples, diluted in water, deproteinised, filtered and pipetted into the reagent. This eliminated errors in the setting of the automatic pipettes used for dilution and deproteinising. It was found that glucose was lost from the standard solution even when stored at $+1^{\circ}C$ and standards were therefore kept for only 2-3 days.

The method was found to be accurate with solutions of glucose in distilled water and the results of the 2 determinations are shown in Table 18.

Initially it was found difficult to achieve repeatable results when whole blood samples were used. This was eventually traced to the high sedimentation rate of porcine erythrocytes. When blood samples were allowed to stand undisturbed, the sample separated out within 30 minutes into two clearly definable layers one consisting largely of plasma and the other largely of erythrocytes. If, before subsampling for analysis, the blood was not thoroughly mixed, the subsamples contained different proportions of plasma which contained nearly all the glucose (Somogyi, 1933; Goodwin, 1956b). When the blood was properly mixed by gentle rolling in the bottle replicates were usually within the accepted standard of less than 2% difference between replicates. To reduce error due to sedimentation, samples for glucose analysis were always just over 2 ml and each replicate subsample was 1 ml.

The recovery of glucose from mixtures of blood and glucose solution was also investigated. Glucose was added to blood samples by diluting the blood in four glucose solutions containing increasing glucose concentration instead of in distilled water as in the normal determination.

The results are shown on Table 19. This degree of recovery was considered sufficient for the purposes of estimating blood glucose

for/

TABLE 18. Recovery of glucose from solution of glucose in water.

Sample	Optical Density		Average	Glucose Concentration	Actual Glucose Concentration	Error %
1	0.158	0.158	0.158	51.6	52.3	-1.3
1	0.157	0.159				
2	0.280	0.280	0.282	85.0	84.5	+0.6
2	0.284	0.284				

TABLE 19. Recovery of added glucose from blood samples.

Sample	Calculated Glucose Concentration mg%	Estimated Glucose Concentration. mg%	Error mg%	Recovery %
blood	-	102.77		
blood + glucose	125.6	124.6	- 1.0	99.2
blood + glucose	148.7	148.4	- 0.3	99.8
blood + glucose	171.6	173.7	+ 2.1	101.2
blood + glucose	194.6	191.8	- 2.8	98.6

FIGURE 14. The normal pattern of change in blood glucose concentration after an intravenous injection of 50 g glucose in the sow.

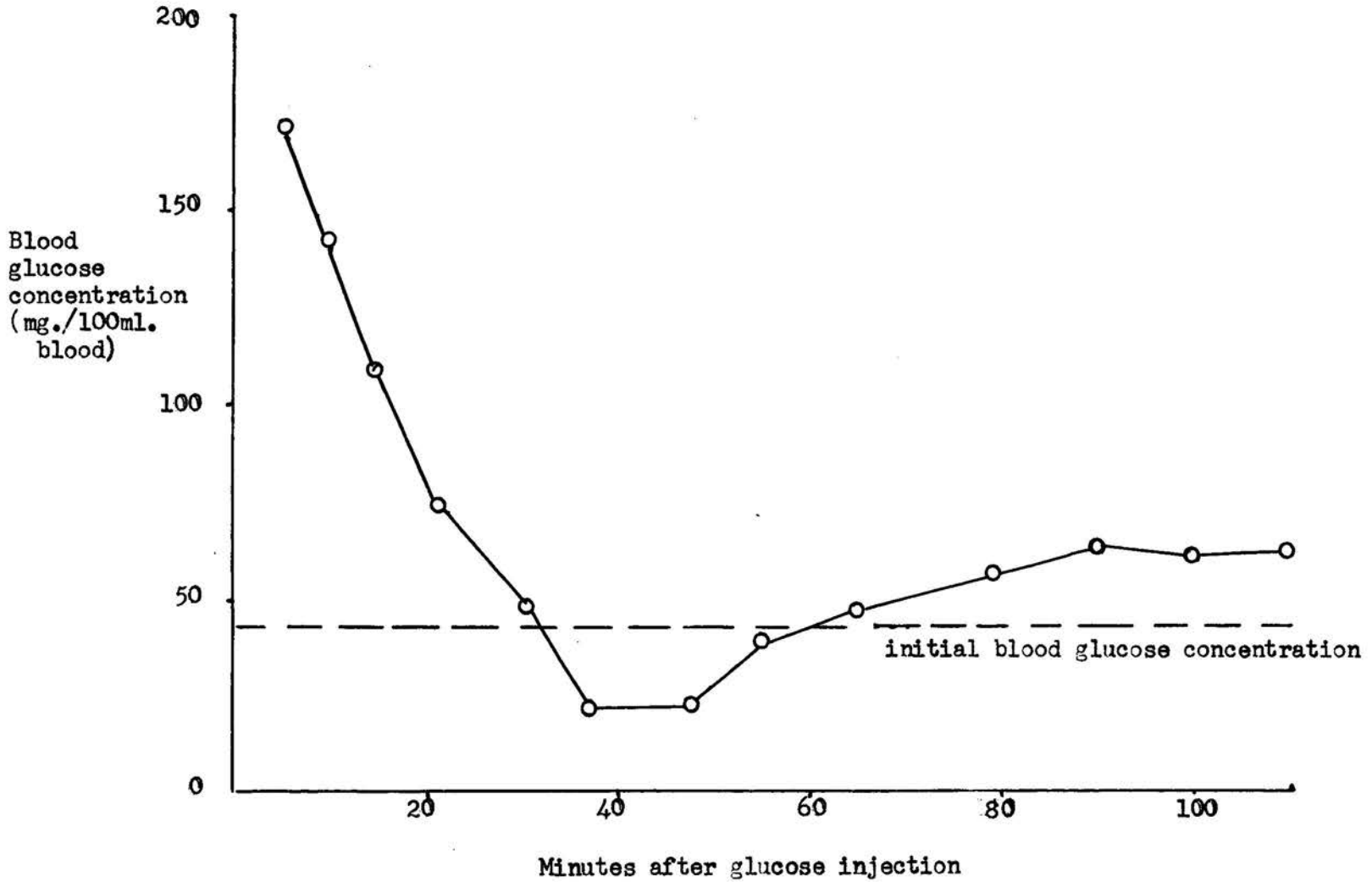

200
150
100
50
0
Blood glucose concentration (mg./100ml. blood)
initial blood glucose concentration
20
40
60
80
100
Minutes after glucose injection

FIGURE 15. **The method which was adopted of interpreting the intravenous glucose tolerance test.**

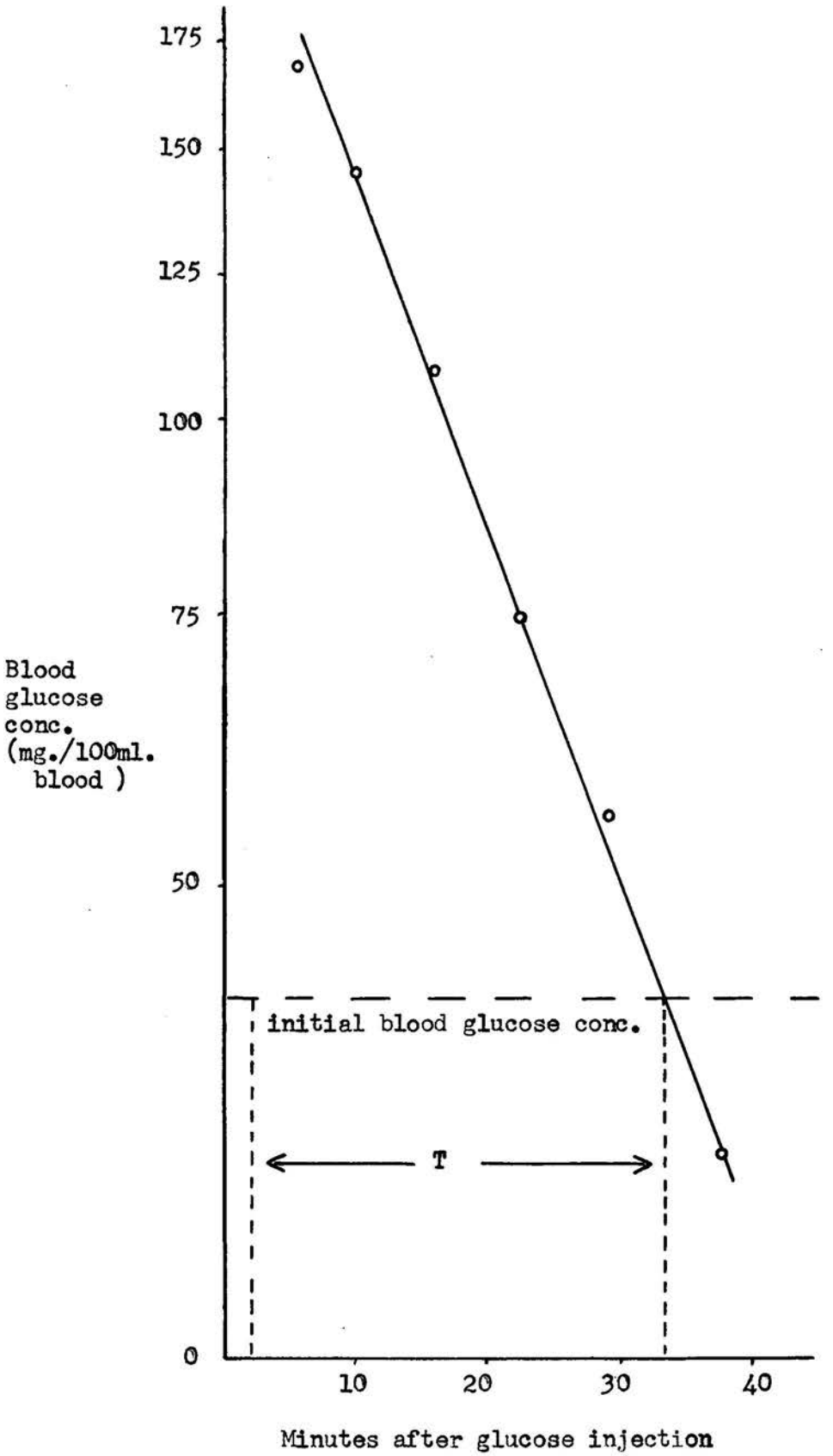
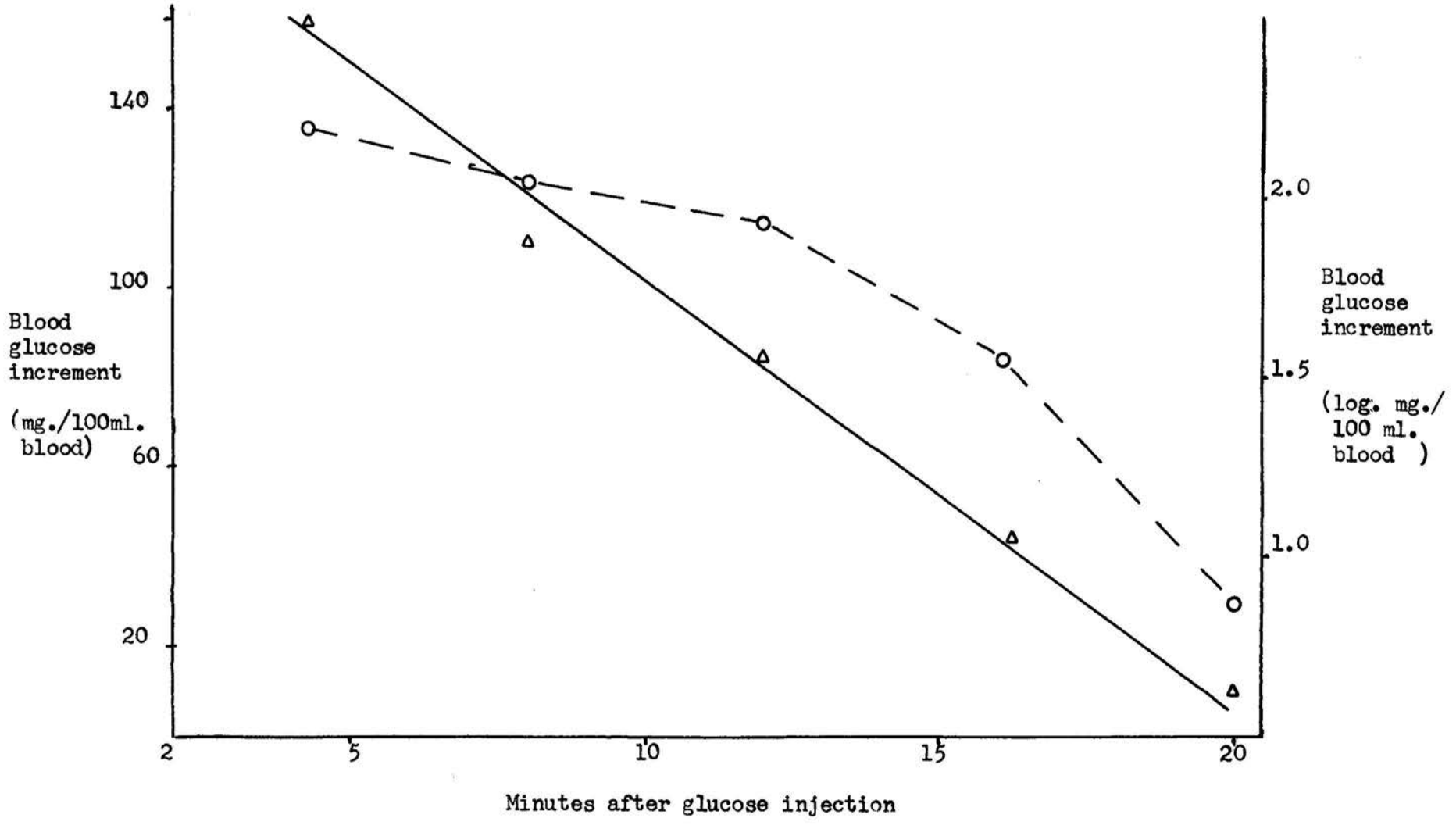

175
150
125
100
75
50
0
Blood glucose conc. (mg./100ml. blood)
initial blood glucose conc.
T
10
20
30
40
Minutes after glucose injection

FIGURE 16. Blood glucose increment and the logarithm of blood glucose increment plotted against time after glucose injection.

o----o the logarithm of blood glucose increment.

Δ blood glucose increment.


140
100
60
20
Blood glucose increment (mg./100ml. blood)
2.0
1.5
1.0
Blood glucose increment (log. mg./100 ml. blood)
2
5
10
15
20
Minutes after glucose injection

for the glucose tolerance test.

Section II. Studies of intravenous glucose tolerance in sows.

a) The animals used. The animals used in this series of experiments were all Large White sows which had been served within the previous 14 days. The nutritional histories of the animals were varied, but they had all been given 2.2 kg. /daily of diet R.S.1., in two feeds, for at least 30 days previous to the tests.

After indwelling catheters had been established in the jugular veins on the day previous to the test, the animals were placed in metabolism crates, where they remained until the experiment was finished.

The glucose tolerance tests were performed exactly as detailed in Section 1a of this chapter.

b) The results and their interpretation. The results of such a test are shown graphically in Figures 14 and 15. Figure 14 shows the pattern of blood glucose concentration after injection of 50 g glucose. Throughout all experiments no animal was found to depart from the general picture of a return of blood glucose concentration to normal within 20 to 40 minutes after injection, followed by a dip below the basal concentration and then a return to a concentration above basal for some time. In Figure 15 Logarithm of blood glucose is plotted against time and it can be seen that the relationship conforms to a linear pattern up to 35 minutes from injection.

Several methods of interpretation of the removal of an intravenous load of glucose were investiaged. Attempts were made to use the Increment index suggested by Duncan (1956), but as shown in Figure 16 the relationship between the logarithm of glucose increment over fasting concentration and time was not linear; indeed over this period the rate of decrease of the glucose increment was almost linearly related to time. The use of the glucose increment as a test of glucose tolerance was therefore discarded. Since the rate of decrease of

the/

the logarithm of glucose concentration was linearly related to time, two measures based on this relationship were investigated. These were, firstly, the rate of decline of the logarithm of glucose concen-:tration (Duncan, 1956), and secondly, the time required for the logar-:ithm of blood glucose concentration to return to the logarithm of fast-:ing glucose concentration (Modified from Tunbridge and Allibone 1940). The rate of decline of the logarithm of glucose concentration was ex-:pressed as the half life and the time to return to fasting concentration in minutes (T). In all cases, two minutes after injection of glucose had commenced was considered as time O.

c) Repeatability of the intravenous glucose tolerance test. A number of glucose tolerance tests were repeated in the same animals after an inter-:val of two or three days, but initially the repeatability was very poor. This lack of repeatability was especially marked when the test was per-:formed at the weekend and it was suspected that this feature was assoc-:iated with a variable fasting interval immediately prior to the test, since such variations would occur at the weekend when the pigs were sometimes fed three hours earlier than usual.

To investigate this possibility the following experiment was per-:formed. Four standard glucose tolerance tests were performed on the same animal after fasting intervals of 15 hours, 15 hours, 22 hours and 8 hours respectively. The results are shown in Table 20.

It can be seen that when the fasting interval was identical then the test repeated well but when the interval was decreased, glucose was removed less rapidly and when the interval was increased the rate of glucose removal increased. Although there is not sufficient evidence to say that the time taken for the blood glucose concentration to return to the fasting concentration after an intravenous load of 50 g glucose is indirectly proportional to the length of the fasting period, there is enough evidence to make a standard length of fast an integral part of the intravenous/

TABLE 20. The effect of fasting interval on the glucose tolerance.

Test	Day	Fasting Interval (hrs)	Fasting Blood Glucose (mg %)	T (min)	Half Life (min.)	Difference in T from Test 1. (%)	Difference in Half Life from Test 1. (%)
1	1	15	53.9	24.46	12.2	-	-
2	4	15	55.9	24.54	11.2	+0.3	-8.2
3	6	22	56.1	19.3	8.07	-21.0	-33.8
4	8	8	50.0	27.0	11.93	+11.0	-2.2

TABLE 21. The repeatability of the intravenous glucose tolerance test.

Test No.	Fasting Blood Glucose Concen-:tration. (mg %)	T (min.)	Half Life (min.)	Difference in T (%)	Difference in Half Life (%)
1	53.6 50.1	27.36 27.34	12.02 14.66	-0.008	+22.0
2	50.0 50.0	32.2 31.5	14.05 12.45	-2.2	-11.4
3	53.9 55.9	24.5 24.5	12.2 11.2	0	- 8.2
4	40.0 46.6	20.7 21.4	9.91 8.50	+3.4	-14.2
5	50.0 46.6	40.6 38.7	17.06 15.71	-4.7	- 8.0

intravenous glucose tolerance test. The length of fast adopted was 15 hours.

When a standard 15 hour fast was incorporated into the test, repeatability was greatly improved, as shown in Table 21.

The degree of repeatability shown in the time required for the log blood glucose concentration to return to the log fasting blood glucose concentration (T) was considered acceptable for a test of this nature. Although the half life of the glucose concentration showed poor repeat-:ability it was not altogether discarded as it was readily obtained from the calculations required to derive T.

d) The effect of the position of the catheter on the results. It was consid-:ered necessary to investigate the effect of the position of the tip of the catheter, which would determine the source of the blood samples, on the results of the test. Three separate experiments were performed to this end.

Experiment A. Indwelling catheters were established in opposite jugular veins, one catheter being 4" nearer the heart than the other. This was considered to represent the maximum difference in position of the tip of the catheter between animals which could occur under normal circum-:stances.

Experiment B. One catheter was established in the jugular vein in the standard way, while a second catheter, established in the opposite ear, was inserted only a few inches and drained little more than the ear tissues.

Experiment C. A catheter was established in the jugular vein in the normal way. In the opposite ear a catheter was established in an artery. This catheter was of a finer bore than the venous catheter and was inserted 14" into the artery. This procedure was adopted to reduce the pressure and flow from the arterial catheter, since catheters of the size used for veins, when inserted into arteries, were difficult to handle be-:cause of the amount of blood discharged.

In all three experiments glucose was injected through the long venous catheter. Fasting and post-injection blood samples were collected/

collected from all catheters. The time taken to return to the fasting blood glucose concentration was calculated for all catheters. The results are shown in Table 22.

These results show that the possible error due to catheter position was slight. It was considered likely that much of the error between the catheters in the above experiments was due to the use of a catheter in either ear, as it was very difficult to sample simultan-:eously from both catheters as the pig would not tolerate being approached from both sides. The error caused by this is apparent in Experiment C where the arterial blood glucose concentration is apparently the same as the jugular vein blood glucose. This might be explained by the fact that the animal was nervous and the arterial catheter fasting sample was obtained first. As arterial catheters are so unpleasant to handle some time was spent on this operation especially in blocking the catheter after samples had been obtained. In the meanwhile the blood glucose concentration had risen due to excitement and hence the venous blood glucose concentration recorded was not completely comparable to the arterial concentration. A true fasting level might be determined by taking both arterial and venous samples over a period of say 15-30 minutes to determine the arterio-:venous difference. Immediately before the glucose is injected a venous sample should be obtained and the arterial concentration calculated from the average arterio-venous difference of the past 30 minutes.

In general it can be said that the intravenous glucose tolerance test performed in adult pigs and interpreted as described here previously is as repeatable as can be expected and is unlikely to be affected by the site of blood withdrawal.

e) Discussion of results. The pattern presented here of the response to an intravenous load of glucose in pigs does not agree with other reports on intravenous glucose tolerance tests in pigs. Hanawalt, Link and Sampson/

TABLE 22. The effect of catheter position on the results of the intravenous glucose tolerance test.

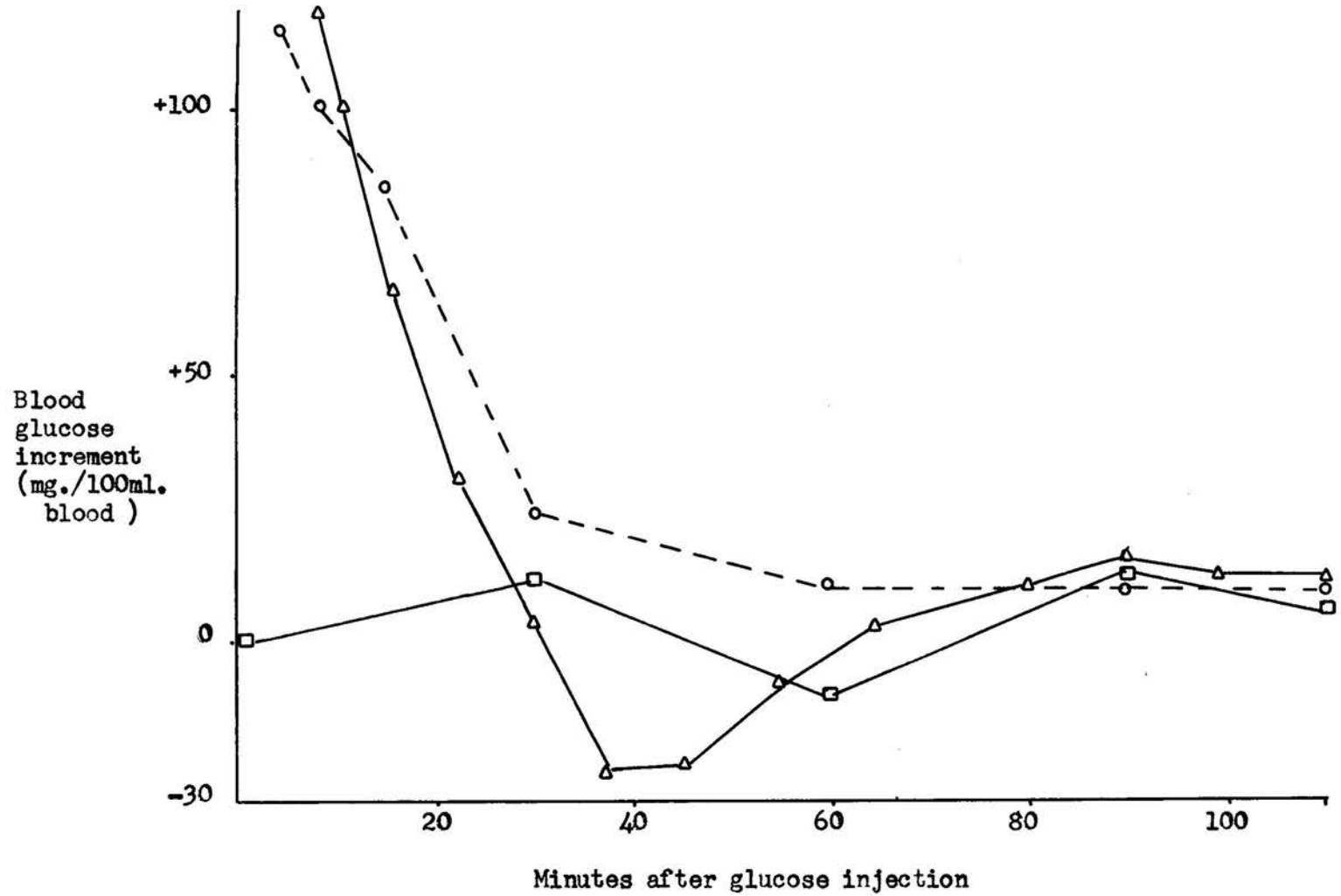
Experiment.	Catheter	Fasting Glucose Concen-:tration (mg%)	T (min.)	Half Life (min.)	Difference in T (%)	Difference in Half Life (%)
A	Jugular	53.9	24.46	10.79	-	-
	Jugular - 4"	52.3	24.21	11.10	-1.0	+2.0
B	Jugular	42.5	32.16	14.00		
	Ear	46.9	33.35	13.44	+3.7	-4.0
C	Jugular	47.5	23.67	8.70	-	-
	Arterial	47.5	24.92	8.71	+5.3	0.0

FIGURE 17. The blood glucose increment in the pig after intravenous glucose injection.

Δ——Δ present study.

O----O Link (1953a).

□——□ Hanawalt et al (1947).


+100
+50
0
-30
Blood glucose increment (mg./100ml. blood)
20
40
60
80
100
Minutes after glucose injection

Sampson (1947) performed tests of this kind in pigs weighing 5-30kg. The glucose was injected in 50% solution at a rate of 0.75 g/kg body-:weight, and a fasting period of 36 hours prior to the test was adopted since an 18-hour fast gave tolerance curves which were "irregular and showed a lag in the rise of the sugar level". The results of the test after a 36-hour fast indicated that the blood glucose on average returned to normal concentrations 60-90 minutes after glucose injection. There was no dip to hypoglycaemic levels, and Hanawalt concluded that "intravenous carbohydrate tolerance curves obtained on healthy pigs are quite similar to those procured on normal human subjects".

The difference between the results of the present study and that of Hanawalt can be resolved by considering the times at which blood samples were taken after glucose injection, and the difference in fasting period adopted. The results obtained by Hanawalt after an 18-hour fast are shown in Figure 17, together with the results of the present study. The glucose concentrations are expressed in mg/100ml increment to allow for the different methods employed to determine blood glucose concentration. It can be seen that by sampling at 30-minute intervals Hanawalt missed the most important part of the response, and by adopting a 36-hour fast, the so-called 'fasting diabetes' was induced with the result that the return of blood glucose values to normal was delayed. This was very clearly demonstrated when the fasting period was altered by Hanawalt to 48 hours. Under these circumstances the fasting blood glucose concentration was depressed and the time taken for blood glucose to return to normal concentrations after an intravenous load was three hours.

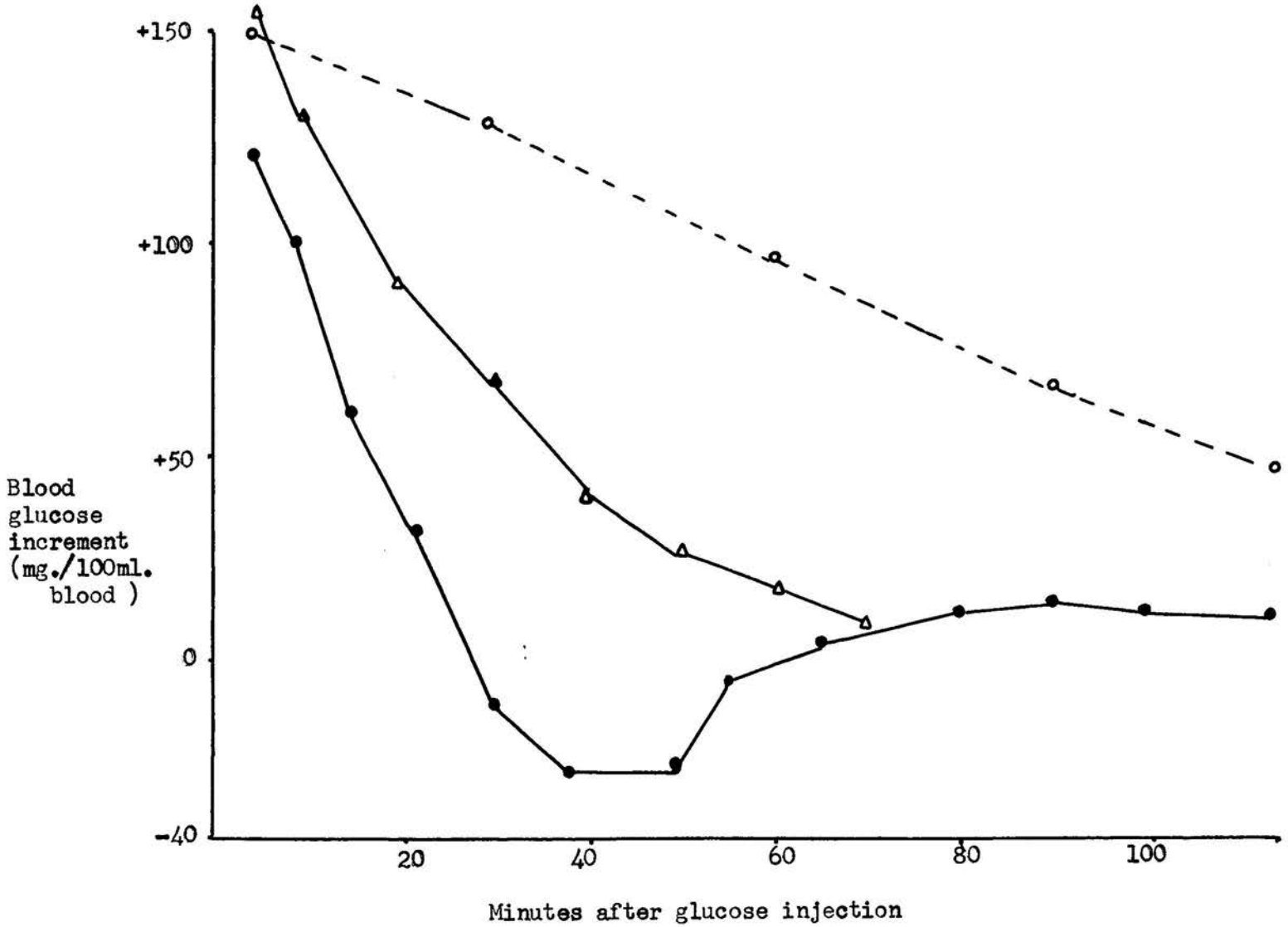
Meyer, Briskey, Hoekstra and Brey (1962) performed intravenous glucose tolerance tests on 'market weight' pigs, employing a fasting period of 36 hours and an injection of 0.75 g glucose/kg bodyweight. The/

FIGURE 18. The change in blood glucose concentration in the human, sheep, and pig after an intravenous dose of glucose of 0.3 - 0.4 g/kg liveweight.

o-----o sheep (Reid 1958).

Δ——Δ human (Duncan 1956)

●——● pig (present study)


+150
+100
+50
0
-40
Blood glucose increment (mg./100ml. blood)
20
40
60
80
100
Minutes after glucose injection

The results of these tests were similar to those of Hanawalt. Meyer and his co-workers who had shown that excitement raised blood glucose, rather surprisingly injected the glucose through the anterior vena cava and removed samples by amputating the end of the tail.

Link (1953) performed intravenous glucose tolerance tests on four young pigs after a 24-hour fast. No details of the quantity of glucose injected are given, and blood samples were again collected from the anterior vena cava. These results are also presented in Figure 17, and show a very similar pattern to the results of the present study. Since no samples were taken between 30 and 60 minutes after glucose injection, the hypoglycaemic phase was, however, missed.

The differences between the reported responses of the pig to intravenous glucose loading are therefore more apparent than real, being a function mainly of the intervals between sampling after glucose injection and probably to a lesser degree, the excitement caused by the various methods of taking blood samples.

The response shown by the pig to an intravenous glucose load is compared in Figure 18 to the response shown by two other species, the sheep and the human, to a similar dose of glucose. It can be seen that the pig removes the excess glucose very much more rapidly than either the human or the sheep. The difference between the pig and sheep is not surprising, the sheep being a ruminant, but the difference between the pig and the human is unexpected as both are omnivorous monogastrics. The difference lies both in the rate of removal and in the hypoglycaemia which develops in the pig. This is unlikely to be due to the use of venous blood in the present study since in Experiment C mentioned earlier in this Chapter, the rate of removal of glucose was almost identical using venous and arterial catheters, and the arterial samples showed the same degree of hypoglycaemia. Indeed, during the/

the period of maximum hypoglycaemia no arterio-venous difference was recorded.

All reported oral glucose tolerance tests in pigs have indicated that the pig is a poor tolerator of glucose (Carlson and Drennan, 1912; Eveleth, 1934; Hanawalt et al, 1947; Bunding, Davenport and Schooley, 1956). This, however, is almost certainly due to a prolonged uptake of glucose from the alimentary canal associated with a delayed gastric emptying time (Dukes, 1942).

Further differences between the pig and human, dog and cat were pointed out by Lukens (1937) who showed that the response to complete pancreatectomy in the pig showed marked deviations from the response shown by dogs and humans. In a series of 9 pigs, Lukens showed that fasting glucose excretion in urine was slight or absent and the fasting nitrogen excretion was low. Ketonuria was marked, but not accompanied by severe acidosis. Pituitary extracts made the pigs' condition resemble human diabetes more closely, and Lukens suggested that the mild 'diabetes' induced by pancreatectomy was associated with some difference in pituitary function. Similar responses to complete pancreatectomy have been reported by Minkowski (1893), Gould and Carlson (1911) and Carlson and Drennan (1912).

This series of experiments has shown that the intravenous glucose tolerance test in pigs is repeatable provided that the fasting period is carefully controlled. It has also been shown that the results obtained from intravenous glucose tolerance tests on pigs in this series of experiments differ only in interpretation from those reported on pigs in the literature. Further evidence has been obtained to indicate that the control of carbohydrate metabolism in pigs differs from that reported in other species.

CHAPTER 10. STUDIES ON THE GLUCOSE TOLERANCE OF SOWS AND THE BIRTH WEIGHT OF THEIR LITTERS.

Section I Experimental Details.

a) Introduction. The aim of this study was to determine whether there was any relationship between the glucose tolerance of the sow and the birthweight of the litters she produced.

b) Materials and Methods. A total of 36 Large White sows were drawn from the Institute herd, over a period of 18 months. The sows included all the animals except First Parity used in the studies on blood volume, described in Chapter 4, and a number of 4th to 6th parity sows. There were in all four 2nd parity sows, six 3rd parity sows, 20 4th parity sows, five 5th parity sows, and one 6th parity sow. The nutritional histories of the animals were as stated in Chapter 4.

The animals were weighed and a catheter established in the jugular vein in the early afternoon. The sows were fed at 6 p.m. that evening and confined to a metabolism crate overnight. On the following morning at 9 a.m. an intravenous glucose tolerance test was performed as described in Chapter 9. Blood samples were always analysed on the same day.

As standard procedure in the Institute, all piglets were weighed individually as soon after birth as possible, including those litters born at the weekend. Thus full details of all litters born before the test were available for all the sows. After the test all litters produced by the sow were similarly recorded until the animal was disposed of. On average records were obtained for four litters per sow and ranged from two to six litters.

c) Statistical analyses. The principal measure of birthweight adopted was the mean of the mean of the mean birthweight for all litters pro-:duced by the sow. To demonstrate this, the case of a sow having produced four litters can be used. In each litter the total weight of piglet/

piglets produced is divided by the number of pigs, alive or dead at birth, to give the mean birthweight. The mean birthweights for all four litters are summated and divided by four to give the mean of the mean birthweight (y_1 kg). A further measure, the mean of the mean birthweight for the first three litters (y_2 kg) was also used.

Two measures of glucose tolerance were used, the time required for the blood glucose concentration to return to fasting level (T mins.) and the half life (x_1 mins.) The liveweight of the sows (x_2 kg) was also included in the analyses.

The analyses performed were:-

(1) Regression analyses of birthweight (y_1) on glucose tolerance (T) and (x_1) and of y_2 on T and x_1 were carried out on all animals and on various subgroups.

(2) Regression analyses of birthweight (y_1) on glucose tolerance (T) and on either liveweight (x_2) or on the product of T and x_2, (x_3).

(3) Analyses of variance were made on the mean birthweights per piglet for individual litters in order to assess the contributions due to variations between sows, between parities and residual.

(4) Regression analyses of total litter weight (z kg) on glucose tolerance (T).

d) Results.

(1) Analyses of the complete results from 33 sows gave the regression equation of mean birthweight on glucose tolerance

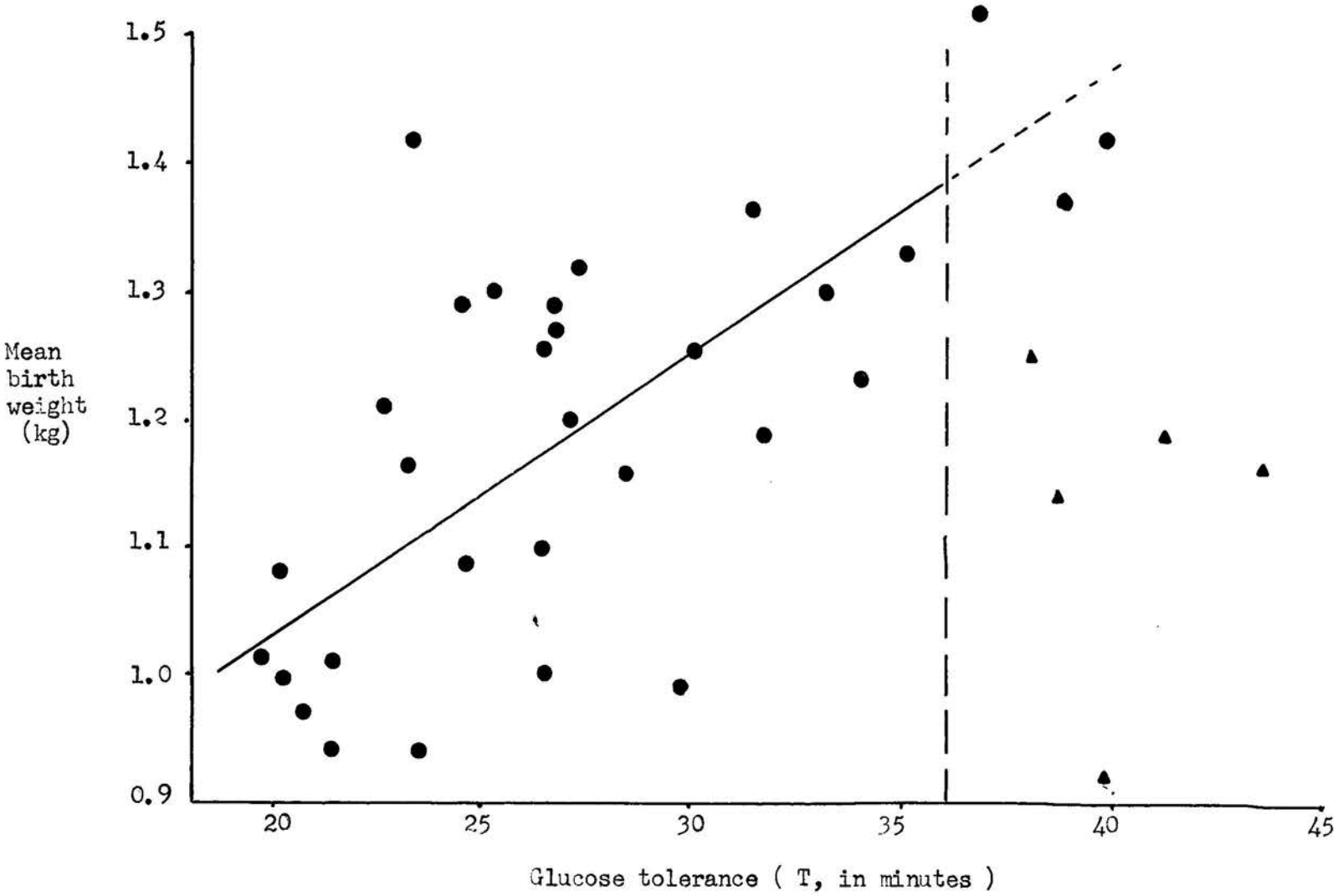
$$y_1 = 0.0075T + 0.962 \quad \text{R.S.D.} \pm 0.141$$

which just failed to be significant at the 5% level. The low order of this relationship was caused by a small number of aberrant points at values of T above 36 minutes. That is, among the animals with poor glucose tolerance some produced heavy piglets, but a number produced piglets smaller than expected. The data were then re-analysed, excluding all values of T greater than 36 minutes, i.e. 3 values where the birthweight

was/

FIGURE 19. The relationship between the sow's glucose tolerance (T) and the birthweight of her litter.

▲ aberrant points where T >36.


Mean birth weight (kg)
1.5
1.4
1.3
1.2
1.1
1.0
0.9
20
25
30
35
40
45
Glucose tolerance (T, in minutes)

was as expected and 5 where it was lower than would be predicted (see Figure 19). The analyses of these 27 values in which T did not exceed 36 minutes yielded the regression equation.

$$y_1 = 0.018\,T + 0.714. \quad R.S.D. \pm 0.123 \quad (T<36)$$

which was significant at the 1% level and accounted for 29% of the total variability in the mean of the mean birthweight (y_1).

On inspection of the data relative to this regression it became apparent that there were two outlying observations one below and one above the line, which were not in accord with the remaining 25 observ-:ations. Their deviations from the line were significant at the 1% level and it was considered that the equation should be re-estimated from the 25 observations alone to obtain an equation free from possible bias due to the two aberrant points.

This analysis yielded the equation

$$y_1 = 0.021\,T + 0.621 \quad R.S.D. \pm 0.098 \text{ kg} \qquad (1)$$

This was considered to give the best description of the data provided that it is not used with values of T in excess of 36 minutes. (Figure 19).

(2) The results from the first series of analysis showed that the mean of the mean birthweight was related to glucose tolerance where values of T did not exceed 36 minutes. It was noticed, however, that those animals which had poor glucose tolerance but lower birthweights than expected were light in bodyweight (< 150 kg) while those which had a poor glucose tolerance and produced heavy piglets were of a high liveweight (>180 kg). On the basis of this observation it was decided to investigate the effect of the sow's bodyweight on the rela-:tionship between glucose tolerance and birthweight. The size of the sample of the population had been increased to 36 sows when this analysis was performed.

Analysis of the data on all 36 sows, including 8 with values of T>36 minutes, /

T>36 minutes, showed that the inclusion of x_3 (the product of glucose tolerance (T) and the liveweight of the sow (x_2) in kg) as well as T appreciably improved the fit over that obtainable using T alone, for the whole range of values of T. The inclusion of x_2 alone was less effective.

The regression equation obtained was

$$y_1 = (0.000066x_2 - 0.0048)\ T + 1 \text{ R. S. D. } \pm 0.135 \quad (2)$$

and accounted for 27% in the total variability in y_1. It should be noted, however, that the R. S. D. is considerably higher than in equation (1).

A further analysis including x_3 was performed using only the data from sows with a glucose tolerance of T<36. No improvement in the prediction of y_1 was obtained by the addition of either x_2 (live-:weight in kg) or x_3 (x_2 X T) to the right hand side of the equation. (3) In the analysis of the group of sows where T<36, comparisons were made of the relative closeness of the relationship when the measures were changed. It was found that T (the time for blood glucose to return to fasting concentration) gave a significantly better indication of birthweight (y_1) than did the half-life in minutes (x_1) P<0.025). The relationship between glucose tolerance and birthweight was slightly poorer when y_2 (the mean of the mean birthweight over the first 3 litters) was used instead of y_1 (the mean of the mean birthweight over all recorded litters) but this difference was not significant.

There was no evidence that relationship between birthweight and glucose tolerance was affected by the parity at which the measurement of glucose tolerance was made. Analyses of variance of mean birth-:weights in the first three parities for 27 sows demonstrated signifi-:cant differences between sows (P< 0.01) and between parities (P<0.05). The differences in parities are confounded with time, and could be explained by alterations in health and other factors as well as by genuine parity effects. Analyses of variance over four parities for 14 sows and five/

five parities for six sows again demonstrated sow differences ($P<0.01$) but no significant parity differences.

From the variability in mean birthweight from litter to litter by the same sow it can be calculated that the standard error of the mean of themean birthweight over four litters is in the order of 0.09 kg per piglet. This establishes a limit to the precision with which y_1 can be estimated by any measure or measurements made on the sow at any one time.

(4) No relationship was found between average total litter weight and glucose tolerance.

Section II. Discussion of the results.

a) The nature of the relationship. These results indicated that there were real differences between the performance of sows with respect to the average birthweight of the piglets they produce, and that these differences were related in part to the glucose tolerance of the sow when measured at the beginning of any pregnancy. Although the inclusion of the liveweight of the sow at the time of the test appreciably improved the fit of a regression equation relating glucose tolerance to birthweight, over the complete range of values of T, it was considered that, as the inclusion of this term did not improve the fit over 78% of the values of T (28 in 36), there may well be no fundamental relationship between liveweight and the correlation between glucose tolerance and birthweight. In the light of this result it was considered unlikely that the use of a standard load, regardless of liveweight, had affected the results.

Although there appeared to be a relationship between glucose tolerance and birthweight, the possibility cannot be ignored that this apparent relationship was an artefact of the particular nutritional treatment applied to lactating sows in the Institute. Himsworth (1934) showed that in humans extremes of diet affected the results of the glucose tolerance test, and in the Institute the feeding during lactation

was related to the litter. However in this instance it was unlikely that this would affect the results for the following reasons.

(1) A number of sows had not been milking for some time before the pregnancy in which the glucose tolerance test was performed.

(2) The relationship between the litter size and the level of feeding during lactation varied both within and between the experiments in which the animals had been used previously.

(3) The animals showing the aberrant results had been fed at a level related to their litters, and not in relation to their glucose tolerance. Therefore if glucose tolerance were related to amount of diet during the previous lactation, these animals would have had a high tolerance of glucose, since the level of feeding during the previous lactation was related to a low litter weight.

(4) During the course of the experiments the herd became infected with virus pneumonia and average birthweights in a number of cases were considerably depressed during the period when the outbreak was most acute. A number of animals were tested immediately after producing a litter in which the birthweight was very much depressed. The estimate of glucose tolerance obtained from these animals correlated with their performance previous to the outbreak of virus pneumonia and to their subsequent performance. For example, sow No. 612 produced in all, 6 litters the average birthweights of which were, from parity 1 to parity 6, 1.34, 1.89, 1.34, 1.40, 0.89, and 1.34 kg., respectively. The glucose tolerance test was performed on this animal at the beginning of the sixth parity immediately after the low-birthweight litter, which was produced at the height of the virus pneumonia outbreak. The glucose tolerance (T = 38.8 minutes) however, when substituted in equation 1, suggested a birthweight of 1.43 kg.

For these same reasons any other effect of the preceeding lactation, such as the possibility of the amount of milk produced by the/

the sow being related to birthweight and also affecting an intravenous glucose tolerance test, can probably be dismissed.

The apparent relationship between the sows' glucose tolerance and the average birthweight of her piglets will be of more immediate importance if the maternal glucose tolerance, or some factor associated with it, influences birthweight, and not vice versa. It seems unlikely that the average foetal weight could affect glucose tolerance, especially since the tests were done in early pregnancy. Furthermore, any foetal influence on maternal glucose tolerance would more probably appear as a relationship between glucose tolerance and total litter weight or number, since these are the two factors most likely to reflect foetal demand for nutrients or foetal output of hormones.

The most obvious way to show which was the causal influence, maternal or foetal, would be to determine whether the glucose tolerance in immature gilts is related to their subsequent performance. An attempt was made to do this, but the experiment was a failure. Twelve maiden gilts were selected and intravenous glucose tolerance tests performed. Unfortunately, immediately prior to the beginning of the experiment, the management of the Institute herd was altered, the dry sows and gilts being fed once a day instead of twice. When the experiment was planned, it was realised that the change in feeding regime might affect the results of a glucose tolerance test, but it was considered that a return to twice-a-day feeding for two or three days, would reverse any effect of one-a-day feeding. The gilts used in this experiment were therefore fed twice a day for three days or more, but, it was found that after a change from once-a-day to twice-a-day feeding, the sow's glucose tolerance decreased for a period of 14 days, and that this decrease was in the region of 20%. The results were therefore hopelessly confounded with the effect of the change in management.

b)/

b) Possible mechanisms by which the birthweight might be affected by Maternal Glucose Tolerance. If the birthweight of the litter is affected by the glucose tolerance of the mother, and not vice versa, then this effect may be brought about in one of two ways; either the birthweight is affected by the sow's ability to tolerate glucose, or glucose tolerance is influenced by the same factor, or series of factors, which act on birthweight.

Some evidence of a possible mechanism was presented by Hoet (1953), who showed that a low dose of cortisone (0.5 mg/day) up to the 19th day of gestation in the rabbit increased both foetal and maternal placental glycogen. After the 21st day of pregnancy cortisone treatment induced no further increase in placental glycogen, but the nutrition of the foetus was "favourably affected". This level of cortisone dosage also caused an impairment of glucose tolerance in a rabbit which was 15 days pregnant. Unfortunately no evidence was presented to show whether cortisone had any effect on the glucose tolerance of the early pregnant rabbit.

A further possible mode of action was suggested by Vallance-Owen and Lilly (1961), who suggested that an insulin antogonist, which was present in the plasma in larger quantities than normal in cases of Diabetes mellitus (Vallance-Owen, Dennes & Campbell, 1958) could cross the placenta. In the pregnant diabetic this antagonist crossed the placenta in sufficient quantity to stimulate the normal foetal pancreas to produce more insulin, to overcome the antagonism. This antagonist inhibited the ability of insulin to promote the uptake of glucose by muscle, but not the uptake of glucose by adipose tissue (Lowly, Blanshard & Phear, 1961). This would explain why the children of diabetic mothers tend to be fatter than normal (Vallance-Owen & Lilly, 1961). Vallance-Owen et al (1958) suggested that in the normal animal, although the antagonist is present, its effect is masked. This would indicate that in the normal animal the glucose tolerance/

tolerance will be unaffected by an insulin antagonist. However, Vallance-Owen & Lilly (1960) considered that the antagonism was mediated through the pituitary-adrenal system and that in pregnancy the antagonism would be increased. It is still doubtful therefore, whether a glucose tolerance test in early pregnancy could indicate normal variations in a factor of this nature.

Baird & Farquhar (1962) have suggested more direct relation-:ship between glucose tolerance and heavy babies born to diabetic humans. In a comparison at birth of the glucose tolerance and plasma insulin-like activity (ILA) between the children of normal mothers and diabetic mothers it was found that the children of diabetic mothers were better equipped to deal with glucose. The plasma ILA 5 minutes after glucose infusion was increased by a factor of 5 in the babies of diabetics and was reduced by a factor of 3 in the babies of normal mothers compared to the pre-infusion level. This difference was reflected in the glucose tolerance of the babies of diabetics, who were able to remove a load of glucose four times faster than the normal babies. Although the normal group consisted of eight infants and the 'diabetic' group consisted of only six, the differences in both glucose tolerance and plasma ILA were significant at the 0.5% and 0.1% levels respectively. On the basis of these results Baird & Farquhar suggested that the maternal hyperglycaemia had stimulated the foetal pancreas to produce more insulin and that this, together with the increase of available glucose, accounted for the increased birthweight of the infants of diabetic mothers.

The theories of both Vallance-Owen & Lilly (1961) and Baird & Farquhar (1962) have a common point, the stimulation of the output of insulin by the B cells of the pancreatic islets of the foetus. Cardell (1953) and Irescoll, Benirschke & Curtis (1960) have shown that the most common finding at post-mortem, of infants of diabetic mothers, is hypertrophy/

hypertrophy of the pancreatic islets, due mainly to the hyperplasia of the B cells. From their histological appearance, these cells seemed to be functionally active, and Cardell (1953) has shown a correlation between the quantity of islet tissue and the birthweight of the baby.

All these theories are limited in their application to the foetal pig as extrapolation from the pathological to the normal state is involved.

Furthermore, it has been suggested that the control of carbohydrate metabolism in the pig differs from that in the human, and it may therefore be unwise to make comparisons between species. On the other hand, it is equally possible that this species difference enables the glucose tolerance test to register normal variations in some important factor in the normal pig, which only become obvious in the pathological human.

c) The body composition of the foetal pig. The theories advanced as to the nature of the correlation between maternal glucose tolerance and birthweight postulate that more glucose may be made available to foetuses which in turn are better equipped to deal with it. Thus the difference between light and heavy pigs is a difference in available energy. As energy can be used either to increase the rate of anabolic processes or be laid down as stored material, then the theories do not absolutely require that there should be differences in the body composition of light and heavy piglets. Widdowson (1950) compared the chemical composition of the lightest and heaviest piglets in three different litters and found that the heavier pig in all cases contained more dry matter, fat and protein on a percentage basis, but mineral content expressed as mg/100 mg was not different. These findings are of very limited value, as they include results from only six animals, and are concerned only with extremes. Furthermore, they are within-litter comparisons, while the present work is concerned with between-litter comparisons. However, as the difference in ether extract was only about 0.3% or 11g of dry matter while the difference in birthweight was on average 100% or 539g fresh material, they do show that differences in birthweight are unlikely to consist/

consist of increased fat content, or to lead to great differences in the percentage composition of the carcass of the pig at birth. One constituent of the dry matter of the baby pig, glycogen, requires particular comment since this storage carbohydrate appears to be present in the foetal pig in extremely large quantities.

Before discussing this material it is essential to point out that glycogen and other carbohydrate material is often estimated by difference, the water, ash, fat and protein being summated and subtracted from the total weight of material, the remainder being the nitrogen-free extract (N. F. E.) which is largely carbohydrate, although not all glycogen. The various chemical methods of estimating glycogen vary in their specificity for glycogen, but it is unlikely that chemical methods would include the products of anaerobic respiration, such as lactic acid, and hence if the animal is killed but glycolysis allowed to continue, chemical methods will underestimate the amount of glycogen and glucose present at death.

McCance and Widdowson (1959) estimated that on a wet weight basis the glycogen content of the skeletal muscle, liver and heart of the newborn pig were 7.2, 8.5 and 1.5% respectively. Brooks, Fontenot, Viperman, Thomas and Graham (1964) estimated the glycogen content of the skinned and boned carcass of the pig at birth to be 6.8% on a wet basis and 31.2% of the dry matter. McCance and Brooks both obtained their results by chemical analysis, although they adopted different methods. Brooks et al also estimated that the glycogen content of the skinned carcass at birth was 7.3% of wet matter or 28% of dry matter. In the same experiment the glycogen content of the liver was estimated to be 11% of wet material or 41.6% of dry matter. These last two estimates were of N. F. E. Elsley (1964) quotes three papers where the glycogen content of the whole carcass was estimated at 10%. 15% and 14% of the dry matter. Curtis, Heidenreich and Foley (1966) analysed the muscle tissue of young pigs at birth and found that glycogen represented/

represented 7-8% of the wet muscle.

It is apparent from the papers reviewed, that in spite of differ-:ent methods of analysis, the pig at birth has a very high content of carbohydrate material, especially in the skeletal muscle. Shelley (1960) compared the glycogen content of skeletal muscle at birth in a number of species and noted that in the sheep and rhesus monkey the glycogen content of skeletal muscle was 30-40 mg/g; all other species examined had less glycogen in the muscle at birth. Thus is would appear that at birth the glycogen content of skeletal muscle in the pig exceeds by a factor of two the content in any other species.

This extremely high concentration of apparent glycogen raises several points, the most obvious being whether there could be an error in all the various methods used to determine glycogen or carbohydrates in the young pig. A concentration of 30% of the dry matter of skeletal muscle is almost unbelievable. However, this figure has been arrived at by at least three different research teams using different methods of direct analysis (McCance & Widdowson, 1959; Brooks et al, 1964; Curtis et al, 1965, 1966).

The presence of high concentrations of glycogen in the skeletal muscles of pigs at birth has some bearing on the present series of experiments on glucose tolerance. In the first instance, it makes it less likely that the explanation of foetal overgrowth in human diabetes put forward by Vallance-Owen and Lilly (1961) can be extended to pigs, since the plasma insulin antagonist described by these authors inhibited the formation of glycogen but not of fat.

d) The significance of the high glycogen content of the pig at birth. The basis of the suggestions relating maternal glucose tolerance to the birth-:weight of the piglets is that energy is limiting to foetal growth in utero and that poor maternal glucose tolerance in some way makes energy more available to the foetuses. However in the last section it was suggested that the newborn pig contained a large quantity of stored energy. This might indicate that there was an excess of energy avail-:able/

available to the foetuses. In the adult animal, energy stores, almost completely in the form of fat, are considered as a means of disposing of an embarrassing surfeit of digested energy in excess of immediate needs (Elsley, McDonald and Fowler, 1964). However, this probably does not apply to the foetal and neonatal animal; indeed, Elsley (1964) suggests that in the pig developing after birth the large increase in fat deposition is an integral part of development. It is equally reasonable to assume that the extremely high content of carbohydrate in the new-:born pig represents a very necessary component of the developing animal, and not just a means of dealing with surplus energy presented to the animal in utero. If this high carbohydrate content is necessary then it must have some function. Shelley (1960, 1961) suggested that the high carbohydrate content of the foetus, largely glycogen, helped it to survive periods of anoxia, especially during parturition. The foetal pig would certainly encounter some periods of anoxia during the long passage through the uterine horn and vagina. Shelley (1964) showed that in the human infants which had died during or shortly after birth, those which had suffered respiratory distress had very much less carbohydrate present in heart, liver and diaphragm than those which had suffered none.

The objection to this supposition is that all the analyses of carbohydrate content have been carried out on pigs after birth. Thus it would be expected that the values in utero immediately before birth would be even higher than those recorded after birth, which is unlikely as the carbohydrate present after birth seems to be exceedingly high.

It has been shown in a number of species (Shelley, 1961) and in the pig (McCance and Widdowson, 1959; Curtis et al. 1966) that tissue carbohydrates decline rapidly after birth, suggesting that they are a source of energy to the newborn animal which must now breathe and feed for itself, and that this carbohydrate is available until lactation has been established in the mother and absorption established from the/

the gut of the newborn pig. McCance and Widdowson (1959) estimated that in newborn pigs starved during the first 24 hours of life, 80% of the dry matter metabolised was carbohydrate. This occurred regard-:less of whether the animal was kept in a warm environment (30°C) or a cold environment (12°C). Goodwin (1957a) showed that the young pig was, unlike the calf or foal (Goodwin 1957b), unable to maintain its blood glucose level within the normal range for longer than 24 hours without food. Once the pigs became hypoglycaemic they soon died. The hypoglycaemia was attributed by Goodwin to the piglet's inability to obtain sufficient glucose from other sources, body proteins and fats, to balance its needs. It was later shown by Mount (1959) that 0_2 con-:sumption /kg liveweight and hence energy requirement increased in starved pigs during the first 24 hours of life.

Tissue carbohydrates have thus two possible functions in the young animal, to compensate for anoxia during parturition and to provide energy for the young animal immediately after birth. The young pig is particularly dependent on tissue carbohydrates as glucon-:ogenesis is not well established until the animal is 2-3 days old, and the general metabolism is closely tied to the concentration of blood glucose (Goodwin, 1957a). As the foetal and neonatal pig appears to be so dependent on carbohydrate reserves it is unlikely that the high carbohydrate content of the body is merely a reflection of a great deal of energy being available to the foetus. In fact it would appear that, regardless of the availability of energy from the maternal organism, a large carbohydrate reserve must be built up <u>in utero</u> by the foetus to enable it to survive the first few hours of extra-:uterine life.

e) <u>The presence of fructose in foetal blood.</u> The extension to the pig of the theory that maternal glucose intolerance induces foetal hyper-:glycaemia, thereby stimulating insulin secretion by the foetal pancreas, might/

might be questioned since it has been shown by Goodwin (1956a) that in ungulates fructose is present in the foetal blood in as great or greater quantities than glucose. After birth the level of fructose declined steadily(Goodwin, 1957b; Shelley, 1960; and Curtis et al 1966). Fructose is not, however, found in the foetal blood of carnivores and rodents (Goodwin, 1956) or primates (Shelley, 1960). Goodwin (1956a) post-:ulated that fructose was present in foetal blood in ungulates because the low maternal blood glucose made retention of glucose by the foetus difficult, and to prevent loss of glucose from the foetal circulation, glucose was transformed to fructose in the placenta (Huggett, Warren and Warren, 1951). The fructose thus formed was retained by the foetus since it did not pass back to the maternal circulation (Alexander, Andrews, Huggett, Nixon and Widdas, 1955). The production of fructose by the placenta was independent of both maternal and foetal blood glucose levels, and ensured that the foetus was adequately protected against periods of maternal hypoglycaemia.

Unfortunately for this theory, further work by Nixon (1963) showed that the sheep's placenta did not prevent fructose passing from foetal to maternal circulation. Previously Andrews, Britton, Huggett and Nixon (1960) showed that the perfused foetal liver was unable to utilise fructose, and that in the neonatal sheep the liver could not do so until the fifth day of life. Alexander, Britton and Nixon (1966) then showed that foetal sheep utilised only a very small quantity of fructose in the tissues. It was also shown by these workers that fructose was not utilised to any great extent even when foetal blood glucose levels were very low. On the other hand, the foetus metabolised glucose readily, and the glucose lost from the foetal circulation, if completely oxidised to CO_2 and water, accounted for almost all the foetal O_2 consumption. The full-term foetuses did not utilise exogenous glucose, and in some cases the blood glucose level continued to rise, especially/

especially when the foetuses were hypoxic. This was presumed to be due to the utilisation of liver glycogen.

This short review of the literature has in no way clarified the function of fructose in the foetal blood, but the work of Alexander et al (1966), Nixon (1963) and Andrews et al (1960) has shown that it is unlikely that fructose is used directly as a source of energy. Thus the theory proposed by Goodwin (1956a) is, in the light of present knowledge, unlikely to be correct. It has, however, been shown that glucose is utilised by the foetal sheep and that the amount of glucose disappearing from the foetal bloodstream could account for almost all the energy used. The dangers of extrapolating to another species cannot be ignored but it is reasonable to assume that the carbohydrate metabolism of the foetal pig is similar.

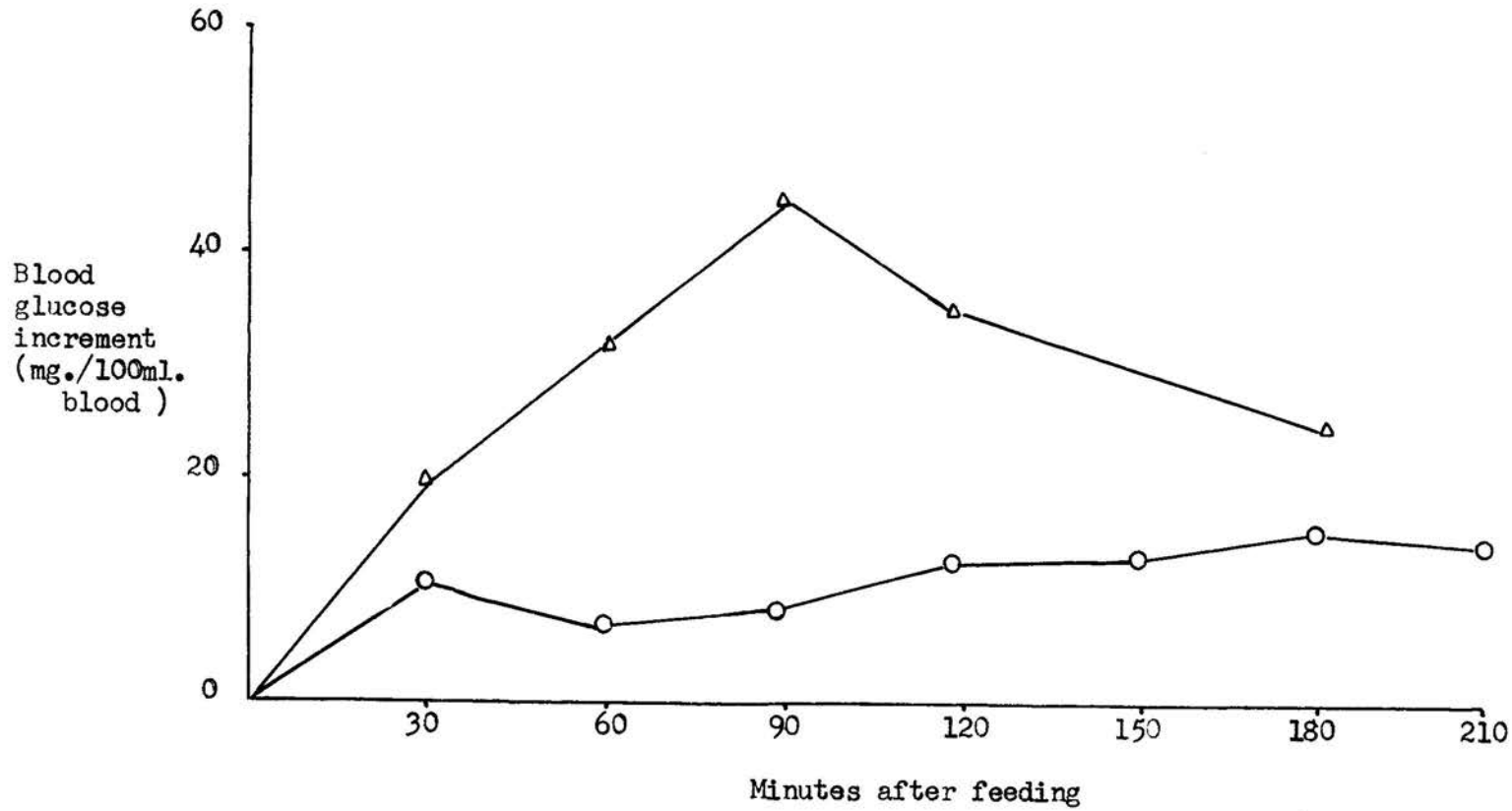
It has been shown that the average birthweight of the piglets produced by a sow is related to the maternal glucose tolerance, a poor maternal glucose tolerance resulting in high average birthweights. It seems probable that the theory of Baird and Farquhar, that diabetes in human pregnancy exposes the foetus to high concentrations of glucose which stimulate the foetal pancreas, could be applied to these findings in the pig. The presence of a high concentration of fructose in the blood of the foetal pig in no way invalidates this theory.

f) The effect of additions of glucose to the diet in late pregnancy. The work reported by Curtis, Heidenreich and Foley (1965) on the effect of additions of glucose to the diet of sows in late pregnancy on the birthweight of pigs tends to support this theory. In the experiment reported by these authors, six sows were given 2.27 kg daily of a standard diet until the 98th day of pregnancy. From the 99th day all sows were given 1.36 kg/100 kg bodyweight of the same ration but were allocated randomly to two treatment groups. One group served as a control while the other received daily an oral load of 1.5 g glucose/kg bodyweight. The birthweight/

FIGURE 20. Blood glucose concentrations in adult pigs after a normal meal and after an oral glucose tolerance test.

o——o blood glucose after 1.1 kg meal (present study)

Δ——Δ blood glucose after 1 - 2 g glucose/kg orally (Bunding et al 1956)


60
40
20
0
Blood glucose increment (mg./100ml. blood)
30
60
90
120
150
180
210
Minutes after feeding

weight of the young pigs produced by the three glucose-loaded sows was significantly higher than the weight of those produced by the controls. The mean birthweight of pigs produced from sows given glucose was 1530 ± 276 g as compared with the weight of pigs from the control sows which was 1390 ± 227 g.

The significant point about this experiment was that it was one of the few recorded instances where a significant effect on birthweight ($P < 0.025$) had been obtained. It is unlikely that the increase in birthweight (10%) was due to the increase in daily energy intake which was approximately 15%, as it is well known that birthweight is affected only by extreme planes of nutrition (Ensley, 1964).

The result, however, is not unexpected in the light of the results of the experiments reported here, and there are two possible explanations for the effect. The first explanation is that the daily dose of 1.5 g glucose/kg liveweight made more sugar available to the foetus since it would cause considerable elevation of the maternal blood glucose. The effect on blood glucose level of the ingestion of 1.1 kg of a standard diet by the sow is shown in Figure 20. These results are the average of the results obtained from five early pregnant sows from the Institute Herd. The effect of feeding 1-2 g glucose/kg of bodyweight as an oral glucose tolerance test is also shown in Figure 20, and is reproduced from Bunding et al (1956). The effect of feeding both sugar and diet together on the blood glucose is unknown, but it seems likely that the blood glucose would at least follow the pattern shown by the sows given glucose orally, and it is probable that the blood glucose would rise even higher than in a normal oral tolerance test. The increased birthweight in the group given 1.5 g glucose/kg bodyweight could be explained by postulating that as a result of the higher glucose levels achieved in the maternal blood more glucose became available to the foetuses.

The second possible explanation is that as an oral glucose tolerance test was performed daily the sow's ability to handle glucose was decreased.

It/

It has been shown by Link (1953a) that daily intraperitoneal injections of 5 - 12 g glucose/kg liveweight in the young pig very rapidly caused decreased glucose tolerance, and in some cases an irreversible diabetic type of condition occurred in 14 days. In the present series of experiments it was found that intravenous loads of 50 g of glucose given at two-day intervals over a period of 14 days caused an increase in glucose tolerance followed by a sharp decline in tolerance. This effect was thought by Link (1953a) to be due to depletion of the pig's supplies of insulin, and it can be postulated that a daily dose of 1.5 g glucose per kg liveweight caused some depletion of the sow's available insulin. This would probably result in a decreased glucose tolerance.

As a result of the decreased glucose tolerance, the sow's blood glucose might remain elevated for a longer period than usual after a meal, resulting in more glucose being made available to the foetuses.

Both these explanations presuppose that an increased gradient between the maternal and foetal circulations will result in increased placental transfer of glucose to the foetal circulation. There is no evidence that this is the case, although Widdas (1952) has suggested that in the sheep at low concentrations of glucose in both circulations, the transfer of glucose across the placenta appears to conform to the laws of simple diffusion. Therfore an increase in the gradient across the placenta would result in increased transfer of glucose. However, he also pointed out that when the gradient between maternal and foetal circulations was large, the differences being 100 mg glucose / 100 ml blood or greater, the transport of glucose appeared to depend on active transport. As a result of this the amount of glucose transported tended to be relatively constant, despite the large gradient in glucose concentrations. Obviously some further information on the transport of nutrients across the placenta would be very useful in the present study.

CHAPTER 11. GENERAL DISCUSSION.

The experiments reported in this thesis were designed to investigate the possibility that in the sow

1) changes in plasma and red cell volumes during the whole of pregnancy

2) the glucose tolerance of the sow in early pregnancy are related to the birthweight of the foetuses produced by the sow. While these studies have indicated a relationship between maternal glucose tolerance and the birthweight of the piglets produced by the sow, they have left several questions unanswered and suggested a number of fresh lines of research. In this Chapter these points will be indicated and possible methods of investigating them suggested.

The method used to measure plasma volume in this study (Evans blue) has given repeatable results, (S. D. $\pm$ 2.56%) which compare well with estimates of plasma volume in pigs made by other authors, when differences in technique are taken into account. However the studies on the disappearance of Evans blue from the plasma reported in Chapter 3 left some doubt as to the accuracy of the method, in that it seemed likely that a portion of the lymphatic volume was being included in the estimate of plasma volume. To elucidate this problem further it will be necessary to compare the estimate of plasma volume made using Evans blue in a series of animals with those made in the same animals using some other marker which is too large to penetrate the capillary walls. The most suitable marker of this nature would be highly purified dextrans or iron dextrans with a molecular weight in excess of 250,000.

The mean volume of red cells / kilogram of liveweight in 1st parity gilts reported in this study (19.7 ml/kg) compares well with the values reported for similar pigs by other authors, who used direct methods of measurement. In Chapter 3 it was suggested that the pig's spleen was able to dilate or contract, thereby altering the haematocrit of jugular blood by removing or adding red cells to the circulation.

The/

The individual estimates of red cell volume made in this study from plasma volume and haematocrit are unlikely to be very reliable since they will have been affected by fluctuations in jugular haematocrit due to excitement and quite unrelated to any change in red cell volume. To overcome this problem it will be necessary to estimate red cell volume in the pig using red cell markers, the most suitable of which is chromium 51.

The difficulties in measuring red cell volume using plasma volume and haematocrit are illustrated by the results of the serial studies on plasma and red cell volumes throughout pregnancy and lactation reported in Chapter 5. In these studies the results of the estimates made of plasma volume showed, in all three animals, that plasma volume declined during the first 40 days of pregnancy and then rose sharply throughout the remainder of pregnancy. This rise in plasma volume continued after parturition until the second or third week of lactation, after which plasma volume declined sharply. The results from each animal showed only slight deviations from this pattern and the general trend was easily distinguished. However, the estimates of red cell volume varied so greatly between estimates in each animal that it was impossible to determine any definite pattern except a rise during pregnancy. This deviation in red cell volume from estimate to estimate was almost certainly due to fluctuations in haematocrit due to excitement.

The work on the changes in plasma and red cell volume during pregnancy indicated that the increases in plasma and red cell volumes during pregnancy appear to be related to the growth of the foetuses, in that the increases in plasma and blood volumes occur exactly in step with the increases in the weight of the foetuses and membranes. Attempts to correlate the increments in plasma and red cell volume during pregnancy with the total birthweight of each litter were unsuccessful. This failure was probably due to the inability to differentiate/

differentiate between alterations in plasma and red cell volumes related to alterations in the weight of some of the sow's own tissues, not directly concerned with pregnancy, and those alterations in plasma and red cell volumes associated with the products of conception. Attempts were made to allow for changes in body composition by measuring the changes in liveweight occurring during pregnancy but this method was unsuccessful and it is likely that studies of body composition during pregnancy will be needed to put the changes in plasma volume and red cell volume into proper perspective. The studies of body composition could be made using either in vivo techniques or by the slaughter and dissection method.

The plasma in the body is only a part of a much larger entity, the total body water, and studies of plasma volume would benefit from parallel studies of total body water and extracellular water. Knowledge of the changes in total body water and extracellular water would be especially useful in studies of the lactating sow. In the present studies the plasma volume was found to increase during the first two to three weeks of lactation, a result which is directly contrary to that reported for the human. In Chapter 5 it was suggested that this difference was a reflection of the different outputs of milk by the two species and that the increased plasma volume in the pig was related to an increase associated with lactation of the hydration of maternal tissue.

The studies on the glucose tolerance of the sow in early pregnancy indicated that there was a correlation between glucose tolerance and the mean of the mean birthweight of all her litters. However this correlation has been demonstrated in only one herd of pigs and it will be necessary to demonstrate the relationship in another herd of pigs to remove all doubts about its significance since it is possible that the management of the herd may affect the relationship.

Further proof that such a correlation does exist could be

obtained/

obtained by demonstrating the existance of some mechanism linking the sows glucose tolerance at the beginning of pregnancy with the growth of the foetuses in utero. It was for example suggested that a possible reason for the relationship was that where the sow was a poor tolerator of glucose, the foetal pancreas was stimulated to produce more insulin which enabled the foetus to grow larger. The validity of this hypothesis might be tested by comparing at birth, the ability of piglets from large and small litters, to secrete insulin in response to an intravenous load of glucose. It would also be worth-:while to extend the experiments of Curtis et al (1965) who gave glucose orally to the sow during the last 15 days of pregnancy and found that the birthweight of the piglets produced by these sows was significantly higher than the birthweight of those piglets produced by a control group of sows which had not received glucose. This experiment could be extended by studying not only the effect of glucose on the weight of the piglets but also the effect of glucose given to the sow on the piglets' ability to secrete insulin, on the sow's glucose tolerance and on the sow's post prandial blood glucose concentration.

The control of carbohydrate metabolism in the adult pig may differ from that in other monogastrics such as the dog and the cat, and any study concerning the carbohydrate metabolism of the pig must take into account the indications of this possibility obtained not only in this study, but also by Lukens (1937) and Link (1953).

It has not, for example, been definately established whether the pig has a delayed gastric emptying time compared with other monogastric animals such as the dog (Dukes 1942). Were the gastric emptying delayed, then the pig's tissues would be protected against sudden influxes of glucose which would have to be dealt with rapidly to prevent the development of hyperglycaemia and the loss of glucose to the urine. If the pig is thus protected against sudden influxes of glucose/

glucose and therefore high blood glucose concentrations, it will have little need of large, readily available, reserves of insulin. Link (1953a) has suggested that the pig has only small reserves of insulin, while Lukens (1937) showed that the pig could withstand complete pancreat-:ectomy relatively well, only moderate hyperglycaemia developing. Lukens also showed that after pancreatectomy, the pig only increased its degradation of protein by factor of two compared with the much higher figures shown by pancreatectomised dogs and cats. This is not altogether surprising since the pig is an omnivore whose energy is normally ingested in the form of carbohydrate, while the dog and cat are carnivores whose energy is normally ingested largely as protein. Thus the normal dog and cat will be much better adapted to breaking down proteins as a source of energy than the pig.

Eventually these possible differences in the carbohydrate metabolism of the pig as compared with other monogastric animals will have to be investigated, not only in connection with studies on the birthweight of pigs, but also as part of more general studies on the utilisation of ingested energy by the pig.

Of the many possible lines of study suggested by this study on the factors affecting birthweight in pigs perhaps the most useful lines to be pursued immediately would be

1) A study of the changes in total body water, extracellular water, plasma volume and red cell volume during preg-:nancy and lactation. Each parameter would be measured independently and almost simultaneously in the same group of mature animals.
2) A study of the ability of newborn pigs to secrete insulin and on the affect on both the sow and the foetuses of glucose given orally to the sow in late pregnancy.

APPENDIX 1.

The composition of diet R.S.1.

Ingredients	(%)
Ground barley	62.5
Ground maize	15.0
Weatings	15.0
White - fish meal	5.0
Extracted soya bean meal	2.5
Vitamin and mineral supplement ψ	+
Analyses (percentage on air dry basis)	
Crude protein (N^{x} 6.25)	14.0
Dry Matter	86.1

ψ Supplement per pound diet: 1.2g. Ca; 0.20g. NaCl; 7.14 mg. Zn; 31.25 mg. Mn; 4.47 mg. Fe; 3.12 mg. Cu; 3125 i.u. vit. A; 223 i.u. vit. D; 1.12 mg. riboflavine; 7.14 mg. pantothenic acid; and 3.57 ug B_12.

APPENDIX 2.

The composition of diet R. S.2.

Ingredients	(%)
Ground Barley	47.5
Ground Maize	15.0
Weatings	25.0
White - fish meal	2.5
Extracted soya bean meal	10.0
Vitamin and mineral supplement μ	+
Analyses (percentage on air dry basis)	
Crude protein (Nx 6.25)	15.9
Dry matter	85.7

μ Supplement per pound diet; 1.12g. Ca; 0.20g NaCl; 7.14 mg. Zn; 31.25 mg. Mn; 4.47 mg. Fe; 3.12 mg. Cu; 3125 i.u. vit. A; 223 i.u. vit. D; 7.14 mg. pantothenic acid; 1.12 mg. riboflavine; and 3.57 ug. vit. B_{12}.

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